The Journal of Allergy

VOL. 14 SEPTEMBER, 1943 NO. 6

Original Articles

CONTACT REACTIONS IN ATOPY

III. CONTACT REACTIONS IN VARIOUS ATOPIC ILLNESSES

MURRAY ALBERT, M.D., AND MATTHEW WALZER, M.D.
BROOKLYN, N. Y.

IN PREVIOUS communications[1, 2] the writers described a type of contact reaction which was observed when atopic subjects were patch tested with oil-free preparations of various common allergens.

The materials employed were prepared by washing the raw allergens with ether for several days until no trace of oil remained. The residues were then dried and pulverized. The powders were incorporated in petrolatum to form 25 and 75 per cent ointments by weight.

Sixteen allergens were prepared for testing in this manner. They included corn, cottonseed, cow epithelium, egg, feathers (duck and goose), flaxseed, goat epithelium, milk, orris root, plantain pollen, silkworm, ragweed pollen, timothy pollen, tobacco, wheat, and wool.

A bit of the 25 per cent ointment was applied to the skin on the outer aspect of the upper arm. This was covered with a 4 cm. square of adhesive plaster, lined in the center with plain cellophane or waxed paper. After an interval, usually forty-eight hours, the test was removed. The site was examined at frequent intervals for a week or more.

Positive reactions usually began within twenty-four to seventy-two hours after the application of the excitant, although earlier or later onsets were occasionally noted. The height of the reaction usually occurred on the fourth or fifth day.

The reaction differed from that seen in allergic contact dermatitis following the application of common contactants such as oils, resins, dyes, metals, etc. In appearance and in development, it also differed from the tuberculin patch reaction. The atopic contact reaction was superficial, sharply circumscribed, and localized to the area of contact with the excitant. It was accompanied by a variable degree of mild pruritus. It consisted primarily of shiny, discrete, minute vesicles. Erythema was an inconstant finding. When present, however, it was

From the Allergy Division of The Jewish Hospital of Brooklyn.

Read at the Semiannual Meeting of the Allergy Division of The Jewish Hospital of Brooklyn, May 22, 1939.

pink, and localized to the vesicular area. A secondary areola rarely occurred. The surface of the lesion was rough and dry, and subsequently underwent desquamation. Pigmentation, hemorrhage, and necrosis were not observed even in the most marked reaction, but a slight amount of induration was occasionally noted.

Histologic study of the reaction revealed marked edema of the cutis with dilatation of the vessels and lymphatics. There was a pronounced cellular infiltration consisting primarily of polymorphonuclear leucocytes, small lymphocytes, eosinophiles, and large mononuclear cells. Hydropic changes were observed in some of the epidermal cells.

Positive contact reactions occurred for the most part in atopic children between the ages of 5 and 12 years, and only occasionally in atopic adults. In supposedly nonatopic adults and children, contact reactions occurred much less frequently. Silkworm and feathers yielded the greatest number of positives. Forty-eight per cent of atopic children reacted to silkworm, while nonatopic children showed an incidence of only 4 per cent. Fifty-one per cent of atopic children and 10 per cent of nonatopic children showed positive contact reactions to feathers.

The contact hypersensitiveness to silkworm, feathers, and other allergens could be induced by single or repeated applications of these excitants to the skin in the form of patch tests. In this manner, the hypersensitiveness was frequently induced in atopic children, much less frequently in atopic adults, and rarely in nonatopic subjects. The flare-up of previously negative test sites, a phenomenon frequently associated with the onset of epidermal hypersensitivesness to oils, dyes, etc. in contact dermatitis (nonatopic), was not observed in the atopic contact reactions. That the oils derived from the original allergens were not the excitants of the atopic contact reactions was demonstrated by the fact that patch tests with these oils failed to produce positive reactions. Dialyzed saline extract of silkworm, when applied by patch tests, elicited the reaction almost as readily as the silkworm ointment.

The contact type of reaction in atopic individuals could not be passively transferred with the serum of the sensitive patient, and therefore is not mediated by the reagin mechanism.

Many patients with marked reactions by intracutaneous testing failed to react by contact test with these allergens. On the other hand, some individuals with negative reactions to atopens by intracutaneous test showed positive reactions to patch tests with ointment preparations of the same allergen. Both types of reactions were frequently elicited in the same atopic child.

Many subjects reacted positively to more than one allergen by contact test. Some showed as many as five positive reactions in the tests with sixteen allergens.

The present communication deals with the results of contact tests with the aforementioned ointments in a series of adults and children suffering from one or several forms of atopic illness, including asthma,

hay fever, eczema, neurodermatitis, and vernal conjunctivitis. A large series of nonatopic individuals were also tested as controls.

In the analysis of results about to be presented, patients were grouped according to their predominating symptom. Although many patients showed several manifestations of allergy, they were listed only once in the tabulations.

CONTACT REACTIONS IN ASTHMA

Thirty-six atopic patients with bronchial asthma were tested by contact with the sixteen common allergens previously listed. In this group were twenty-eight children and eight adults, varying in age from 2 to 51 years. Many of these subjects showed positive reactions to various common excitants by intracutaneous testing, while a few gave negative reactions to all such tests.

Among the asthmatic subjects, positive contact reactions were obtained with cottonseed, feathers, goat epithelium, orris root, ragweed pollen, silkworm and tobacco (Table I). Among the twenty-eight children, twenty-one (75 per cent) showed one or more positive contact reactions (Table II). In the adult group consisting of eight subjects, only one (12.5 per cent) positive was obtained. The latter reaction was obtained with goat epithelium.

The results listed in Table I show that, among the asthmatic children, the highest incidence of positive reactions was obtained with silkworm ointment. Feathers ranked next in frequency. A few of the children with positive contact reactions showed no definite positive reactions by intracutaneous testing with saline extracts of the allergens. Seven of the twenty-one children with positive contact reactions reacted to more than one allergen.

CONTACT REACTIONS IN ATOPIC ECZEMA AND NEURODERMATITIS

Sixteen children and two adults suffering from eczema and neurodermatitis were tested by contact with the sixteen allergens listed above. The children varied in age from 14 months to 12 years. The two adults were 17 and 20 years of age, respectively. Neither of the adults manifested any positive contact reactions. Among the children, reactions were obtained with cottonseed, feathers, goat epithelium, orris root, silkworm and wool (Table I). Of these allergens, silkworm gave the greatest number of positive reactions. Feathers and orris root also showed a high incidence of positives, but the number of subjects tested with these allergens was relatively small. Ten of the sixteen children in the group (62.5 per cent) showed one or more positive contact reactions (Table II).

It was interesting to note that not one of the eleven children in this group who were tested by contact with egg, milk, and wheat showed a positive contact reaction to these foods, which are generally conceded to be among the most common etiologic agents of eczema. Intracutaneous testing with saline extracts of these allergens usually results in a high incidence of positive reactions in these cases.

TABLE I

RESULTS OF CONTACT WITH INDIVIDUAL ALLERGENS IN ATOPIC ILLNESSES

DIAGNOSIS	ASTHMA		ECZEMA AND NEURODERMATITIS		HAY FEVER		VERNAL CATARRH		TOTAL NUMBER TESTED	TOTAL NUMBER POSITIVE	PER CENT POSITIVE
ALLERGEN	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE			
Corn	0	0	6	0	3	0	6	1	15	1	6.6
Cottonseed	16	3	8	1	20	0	12	1	56	5	8.9
Cow epithelium	11	0	9	0	12	1	10	0	42	1	2.3
Egg	12	0	11	0	3	0	5	0	31	0	0
Feathers	11	5	5	2	11	6	11	4	38	17	47.3
Flaxseed	3	0	4	0	4	0	4	0	15	0	0
Goat epithelium	17	1	11	2	9	2	16	3	53	8	15.0
Milk	12	0	12	0	2	0	5	0	31	0	0
Orris root	13	2	7	2	16	2	10	0	46	6	13.0
Plantain	6	0	6	0	10	0	11	0	33	0	0
Ragweed	13	1	7	0	25	5	11	2	56	8	13.7
Silkworm	33	18	18	6	44	10	18	9	113	43	38.0
Timothy	12	0	5	0	15	1	9	0	41	1	2.4
Tobacco	10	1	6	0	6	0	8	0	30	1	3.3
Wheat	12	0	11	0	9	0	7	0	39	0	0
Wool	16	0	15	0	9	1	13	0	53	2	3.7

TABLE II

SUMMARY OF RESULTS OBTAINED WITH CONTACT TESTING IN VARIOUS TYPES OF ATOPIC ILLNESSES

DIAGNOSIS	CHILDREN			ADULTS		
	NUMBER TESTED	NUMBER POSITIVE	PER CENT POSITIVE	NUMBER TESTED	NUMBER POSITIVE	PER CENT POSITIVE
Asthma	28	21	75	8	1	12.5
Eczema and neurodermatitis	16	10	62.5	2	0	0
Hay fever	20	12	60	28	2	7
Vernal catarrh	16	12	75	2	0	0

CONTACT REACTIONS IN HAY FEVER

Twenty children and twenty-eight adults with pollinosis were tested by contact with the sixteen available allergens. The ages of the patients ranged from 5 to 58 years. Positive reactions were obtained with cow epithelium, feathers, goat epithelium, orris root, timothy pollen, ragweed pollen, silkworm, and wool (Table I). The incidence of positive reactions with ragweed pollen was higher in this series (20 per cent) than in the other atopic illnesses. Feathers (54 per cent) and silkworm (22 per cent) showed a higher incidence of positive reactions than any of the other allergens including the pollens. Of the twenty children tested, twelve (60 per cent) showed one or more positive reactions, while of the twenty-eight adults in this group, only two (7 per cent) showed positive contact reaction (Table II).

A few patients who gave positive contact reactions to ragweed were patch tested with ragweed oil. No positive reactions were elicited with this fraction of the pollen.

Positive contact reactions to ragweed occurred only in individuals with positive cutaneous reactions to ragweed. All excepting one of these patients were clinically sensitive to this pollen.

A contact reaction to ragweed was induced in only one subject by repeated contact with ragweed ointment. This occurred in a child who had been clinically sensitive to ragweed and showed a positive reaction to an intracutaneous test with this allergen.

The single positive contact reaction to timothy pollen among the forty-one cases tested occurred in a hay fever patient with positive reactions to timothy and ragweed by intracutaneous test. This individual, however, manifested clinical symptoms only during the ragweed season.

CONTACT REACTIONS IN VERNAL CATARRH

Eighteen patients with vernal catarrh were studied. Sixteen were children who ranged in age from 4 to 14 years, and two were adults, 33 and 41 years of age, respectively. The patients included in this group had typical lesions of the palpebral or limbus types. Their ocular secretions were usually stringy and contained eosinophiles. The eye symptoms in these cases usually consisted of severe itching, lacrimation,

photophobia, and congestion. These symptoms were usually more acute in warm weather. In most instances, the onset of symptoms preceded the major pollinating seasons, and the acute exacerbations could not be correlated with periods of intense pollination.

Among the eighteen cases with vernal catarrh, positive contact reactions were obtained with corn, cottonseed, feathers, goat epithelium, ragweed pollen, and silkworm (Table I). Twelve (75 per cent) of the sixteen children showed one or more positive contact reactions (Table II). The two adults showed none. The greatest number of positives in this group was obtained with silkworm (50 per cent) (Table I). Feather reactions were positive in 36 per cent of the cases tested. Eight children showed positive contact reactions to silk, although intracutaneous tests with saline extracts of the same allergen gave negative reactions. Some cases of vernal catarrh showed no positive cutaneous reactions by intracutaneous test. The incidence of positive contact reactions among these cases was lower than among those with positive cutaneous reactions.

DISCUSSION

The writers have described a distinct type of contact reaction which occurs almost exclusively among atopic individuals, particularly children, and is elicited by application to the skin of oil-free allergens. This contact reaction seems to occur with almost equal frequency among the various common forms of atopic illnesses.

Contact reactions occur no more frequently in allergic conditions in which the skin is the shock organ (atopic eczema), than in those in which the mucous membranes are the seat of the reaction (hay fever and asthma). The fact that contact reactions occur as frequently among children with vernal catarrh, as among those with hay fever, asthma, and atopic eczema, is additional evidence that vernal catarrh is an atopic illness.

Reactions elicited by the atopic contact test more closely resemble the lesion of vernal catarrh than those produced by the intracutaneous test, or by the patch test of contact dermatitis (nonatopic). This fact encouraged the writers to investigate a series of patients with vernal catarrh by means of contact tests. Tests with the above-mentioned sixteen excitants have not as yet disclosed an etiological relationship between the allergens giving positive reactions and the vernal catarrh. The scope of the testing, however, is being broadened to cover other possibilities. These studies will be reported in greater detail at some future date.

While contact tests have been performed with only a few foods, this type of allergen does not appear to give positive contact reactions as frequently as do the environmental allergens. Wheat, milk, and eggs, the commonest food allergens in atopic illnesses, did not elicit a single contact reaction in fifty-three tests on atopic children. Two of the eighteen contact tests performed with corn resulted in positive reactions.

There did not appear to be any pronounced tendency for the positive contact reactions to be grouped among biologically related allergens, as is frequently the case among reaginic reactions. The data, however, are as yet too meager to permit any conclusions to be drawn on this point.

Another finding of interest was the fact that the contact reactions to pollens occurred more frequently among pollinosis cases than among patients with other allergic symptoms. Also worthy of note was the greater incidence of contact reactions among patients with reaginic reactions than among those showing no positives by intracutaneous test. The interpretation of these findings must await further study of these problems.

SUMMARY

1. Contact tests were performed with oil-free preparations of sixteen common allergens, including foods as well as inhalants.

2. One or more positive contact reactions were obtained in 75 per cent of the atopic children with asthma, in 75 per cent with vernal catarrh, in 62.5 per cent with eczema and neurodermatitis, and in 60 per cent with hay fever.

3. Silkworm and feathers gave the highest number of positive contact reactions with almost equal frequency among the various atopic illnesses. Reactions to pollens tended to occur more frequently among patients with hay fever and vernal catarrh than among those with other allergic conditions. Allergenic foods rarely gave positive contact reactions.

4. The high incidence of contact reactions in vernal catarrh is suggestive evidence of the atopic nature of this illness.

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2. Albert, M., and Walzer, M.: Contact Reactions in Atopy. II. The Incidence of Contact Reactions With Various Allergens, J. Invest. Dermat. **3**: 119, 1940.

ELECTROPHORETIC STUDIES ON THE CHEMICAL FRACTIONATION OF RAGWEED POLLEN EXTRACTS

JOHN M. NEWELL, D.SC.
JAMAICA PLAIN, MASS.

ELECTROPHORESIS, or the migration of charged particles under the influence of a current of electricity, can be used for the qualitative and quantitative study of proteins and similar substances in solutions. An apparatus for this purpose has been developed by Tiselius, and its construction and use have been described adequately in the literature.[1, 2] The apparatus is so designed that the migration of electrically charged particles in the solution is observed by optical means and recorded by a special camera. On the film the components of the solution are represented by one or more dark peaks against a light background. At the start of the electrophoretic analysis these peaks are merged into one fine dark line, but after the current is turned on the line broadens and gradually breaks up into the characteristic peaks. These move and spread out at a rate which depends on the charge and diffusibility of the components. If allowed to stand, diffusion is completed and the peaks fade into the base line. Photographs may be taken at given intervals, and the rate of migration of the particles in the solution can be calculated from the change in position of the peaks. In some cases where a correction can be made, or where the components have essentially the same refractive indices, as in the serum proteins, the quantitative proportion of each component in the solution can be calculated from the areas of the peaks. If the substance under examination is chemically pure, only a single peak should appear. However, one cannot claim that a single peak proves the purity of a substance since it may be formed by the merger of several peaks. On the other hand, however, the presence of several peaks in the picture indicates that the solution contains a number of separate substances. This method, therefore, provides evidence, which is not obtainable in any other way, of the composition of many biologic materials composed of large molecules.

Except for the work of Abramson, Moore, and Gettner,[3] who studied pollen extracts and found several components, some of which they isolated electrophoretically, there have been no determinations of the purity of the products obtained in the chemical analysis of pollen. The electrophoretic method requires much equipment which is not generally available. If chemical methods could be correlated with it, the means for the purification of the pollen-active substances might be mapped out.

From the Allergy Clinic and Laboratory, Massachusetts General Hospital. Supported by a grant from the Eleanor Husted Leal Fund for the Study of Asthma.

The first step in attacking the problem was to study thoroughly dialyzed extracts of whole, low ragweed pollen in the Tiselius apparatus. This differs from the procedure of Abramson, Moore and Gettner[3, 4] who purposely did not dialyze their extracts. Since the charges on the particles in solution, which determine the patterns of the peaks, depend on the hydrogen ion activity, the first experiments were carried out at two pH values. Twenty-five grams of pollen were defatted with petroleum ether in a Soxhlet extractor. The defatted pollen was extracted with 250 c.c. of distilled water for one hour, centrifuged, and the yellow supernatant was dialyzed against running distilled water in a cold room at 5° C. overnight. The dialyzing sac was then allowed to stand in a cylinder of distilled water for four hours, at which time the outer liquid gave no test for salt with a few drops of silver nitrate solution. The inner liquid was then put in a liter balloon flask, frozen in dry ice and alcohol, and dried in vacuo by the lyophile method. A fine yellowish flaky material, which powdered readily and floated on the slightest breeze, was obtained. Part of this was dissolved in a sodium acetate buffer at pH 4.0 and another part in a potassium phosphate buffer at pH 7.0, both solutions having an ionic strength of 0.05. The compositions of these solutions were determined by consulting charts prepared for the purpose by Arda Green.[5] The solutions were further dialyzed against 1500 c.c. of their respective buffers overnight in the refrigerator before examination in the Tiselius apparatus.

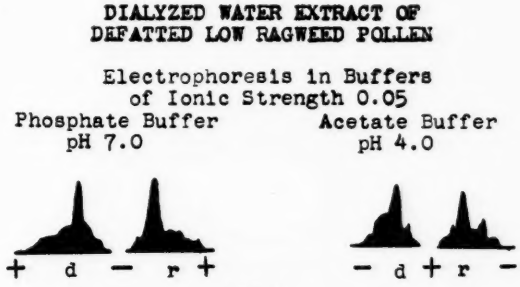

Fig. 1.

Figs. 1 to 4 are tracings of the original photographs. In Fig. 2 the solution under examination was so dark that some peaks did not register in the photograph, but they could be observed visually so they were drawn in the illustration with dashed lines.

The descending (d) and rising (r) sides of the electrophoretic patterns are shown, as well as the charges (+) and (−) of the electrodes toward which the substances in the solutions are migrating.

At pH 7.0 the color of the solution was light brown. When examined in the apparatus at least four peaks could be observed, although three of these soon became broad and ill defined (Fig. 1). The rates at which these traveled were computed in centimeters per second per volt per centimeter of potential. Under ideal conditions the rising and descending sides of the electrophoresis cell should be mirror images of each other, but due to electrical disturbances, the descending side, where the charged particles are moving into the more concentrated solution, is not

as sharply defined as the rising side, where the movement is into the buffer solution. In this paper, therefore, the calculations have been made for the rising side only, even though there are theoretical objections to depending on these values.[6] Two of the peaks, representing negatively charged (acidic) substances, had mobilities of 4 and 8.5×10^{-5}, and a small indefinite one which represented positively charged (basic) material was seen, but could not be distinguished well enough to measure. These moved in the directions of the electrodes of opposite charge, leaving behind a large amount of material which was practically stationary. This observation is essentially in agreement with the observations of Abramson, Moore and Gettner,[3, 4] though they did not report the positively charged component. Since the salt concentration was different, and those workers used undialyzed extracts, it is not possible to correlate the negatively charged substances in these two studies except for the fact that in both instances they were associated with the color of the solutions. The large colorless stationary peak is the material described by Abramson as "USD" or "artefolin."

At pH 4.0 (Fig. 1) a good portion of the pollen extract was insoluble, a fact which has been reported before.[7] At this pH value four peaks were observed also. The relationship of these to the ones observed at pH 7.0 has not been determined, but, although the pictures are similar, the charges on the materials are reversed. The largest amount of material is again found to be without charge, and the colorless substances having positive charges move away from this with mobilities of 3.2 and 5.2×10^{-5} respectively. The light brown color was associated, in this instance, with a small amount of negatively charged material having a mobility of 2.3×10^{-5}.

As will be seen later, the further fractionations of the extracts showed other components to be present which are either formed from the ones in the whole extract or covered up in the electrophoretic pattern.

After the general picture of the whole extract had been studied, attention was given to the composition of the extracts prepared by the extraction of pollen with concentrated solutions of potassium phosphate at pH 7.0. The reason for choosing this medium for extraction is that the salting out effect in it is practically the same as in ammonium sulfate, and, because of the buffering action of the phosphate, a very exact control of the pH of the solution is possible.[8]

The extraction was started with a 4 molar solution of phosphate at pH 7.0 (545 Gm. of KH_2PO_4, 60 per cent converted to K_2HPO_4 by the addition of 2.4 mols or 240 c.c. of 10 molar KOH and made up to one liter of solution). This removed a great deal of dark brown-colored material which was inert when tested in the clinic by scratch tests on ragweed sensitive persons. The first fraction was not studied further, but the extracts in 2 molar phosphate, also at pH 7.0 (272 grams KH_2PO_4, 64 per cent converted by the addition of 1.28 mols of KOH and made up to one liter as before) and in distilled water, were pre-

cipitated as the next higher phosphate concentration by placing the solution in Visking tubes and dialyzing these, while rotating, in several changes of the phosphate solutions as described by McMeekin.[9] The precipitates were examined by electrophoresis after they had been dialyzed free from excess salts and into the proper buffer solutions at pH 7.0 and ionic strength 0.05. Both of these latter fractions contained skin test-active material.

The pictures obtained from them were obscured by the large amount of dark pigment which remained in the solutions (Fig. 2). The colorless stationary component was prominent, as well as the negatively charged components associated with the color at pH 7.0. The extract made with the 2 molar phosphate also showed two positively charged minor components having mobilities approximating 3×10^{-5}. No study was made at pH 4.0 because of the darkness of these solutions.

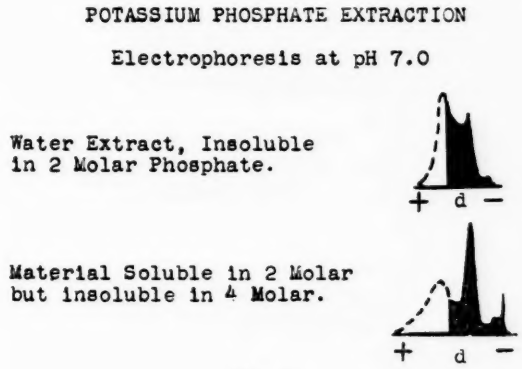
POTASSIUM PHOSPHATE EXTRACTION
Electrophoresis at pH 7.0
Water Extract, Insoluble in 2 Molar Phosphate.
+ d −
Material Soluble in 2 Molar but insoluble in 4 Molar.
+ d −

Fig. 2.

An attempt was made to purify these fractions further to obtain better solutions for examination. They were dialyzed against batches of alcohol of increasing strength in a cold bath kept at minus 5 to minus 15° C. This resulted in finding a large amount of skin test-active substance soluble in 40 per cent (by volume) alcohol. Stull and others[10] had also found active material soluble in high concentrations of alcohol.

This appeared to be a lead worth investigating. Consequently 50 grams of pollen were defatted by extraction with petroleum ether, and extracted in the cold, as before, with 80 per cent (by volume) alcohol. The extract was centrifuged off in a cold room, and the pollen residue extracted in successive steps with 60, 30, and 15 per cent alcohol and, finally, by distilled water. The extracts were precipitated by dialyzing them up to the next higher concentration of alcohol, and filtering off the liquid by means of a sintered glass filter lowered into the liquid on the end of a rubber tube. The precipitates were very difficult to handle because they clogged the filter. In the case of the 15 per cent alcohol and water extracts, the difficulty of filtering was so great that ammonium acetate was added to make a final concentration of 0.005 molar. This

aided in precipitating the suspension, and facilitated the filtration. The precipitates were dissolved in a few cubic centimeters of distilled water and dried in a lyophile apparatus.

The 80 per cent alcohol extract consisted of a varnishlike material which dissolved readily in strong alcohol but was almost insoluble in water. It had no appreciable skin test activity. The 60 per cent extract was clear although it had a faint brown color. It was very active by skin test and corresponds to the early "A" substance of Stull.[10] In the Tiselius apparatus, at pH 7.0 (Fig. 3), it was found to be composed mainly of the stationary material and the two minor positively charged components with mobilities 2.8 and 3.9 $\times$ 10^{-5}. It had almost

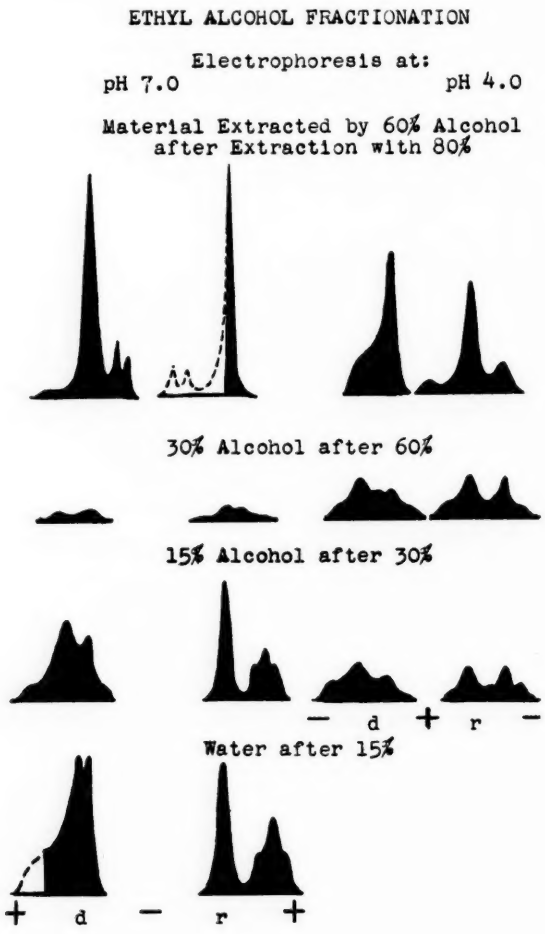

Fig. 3.

none of the negatively charged materials found in the other fractions. However, its composition was by no means simple, a fact which was plainly demonstrated when the electrophoresis was carried out at pH 4.0.

The other alcohol fractions also contained much active material as shown by scratch tests. They, too, contained the stationary component at pH 7.0 which is present in every fraction studied. In addition to this, there were other negatively charged substances with mobilities of approximately 3.3 and 4×10^{-5} and an obscure faster one, but the patterns were so unsymmetrical that the interpretation of them is difficult. Because of this obscurity, the fractions were reworked by dissolving in alcohol of the strength used to extract the fractions, followed by reprecipitation at the next higher concentration. When this was done, the fraction extracted in distilled water did not reprecipitate at 15 per cent alcohol concentration, and so it was combined with the 15 to 30 per cent fraction. When studied at pH 4.0 (Fig. 3) the stationary peak was relatively larger in the higher alcohol concentrations. A positively charged component having a mobility of 3.8×10^{-5} was present in all; but in the precipitate from 30 per cent alcohol, there was a fast moving, positively charged, component having a mobility of 5.5×10^{-5}, which was not present in the other fractions.

Qualitative tests for carbohydrates were made on the reworked fractions by means of the Molisch reaction. The fraction separated by 60 to 80 per cent alcohol gave a very strong reaction, and the fraction separated between 15 to 30 per cent showed only a faint reaction. The other two fractions, 30 to 60 per cent alcohol, and that soluble in 85 per cent, gave intermediate reactions.

Finally, a study was made of the ammonium sulfate fractions separated at pH 4, following the general plan of Stull's work.[7] In the final experiment, 100 grams of pollen was defatted with petroleum ether, and then extracted with distilled water four times with 250 c.c. portions. After the last extraction the combined solution amounted to 850 c.c. It was then dialyzed in the cold against an acetate buffer at pH 4.0, 0.05 molar in sodium acetate. A heavy precipitate and some scum formed, which was removed by centrifuging and filtering. The filtrate was dialyzed against saturated ammonium sulfate containing an excess of the solid to insure complete saturation. The ammonium sulfate solutions had been adjusted to pH 4 with dilute sulfuric acid. Small portions of them were tested with bromphenol blue until the proper pH value was obtained. The dialysis was performed at 5° C. and was allowed to proceed overnight. At the end of that time a heavy precipitate had formed. This was centrifuged down and the precipitate was taken up in 200 c.c. of distilled water and dialyzed again to pH 4.0 in the acetate buffer. The insoluble precipitate which formed was collected, and the supernatant was set to dialyzing against saturated ammonium sulfate at pH 4. This was repeated fifteen times before all the precipitate would dissolve in the acetate buffer. Even then, in subsequent operations, insoluble precipitates would occasionally be found. An electrophoretic pattern was obtained from this material after the fifteenth precipitation, which showed the main stationary component

and four minor positively charged ones at pH 4.0 (Fig. 4), two being close together at about 4×10^{-5}, and the others having mobilities of 5.5 and 6.7×10^{-5}.

The soluble precipitate after fifteen reprecipitations was still approximately as active by skin test as the original extract in the same dilution,

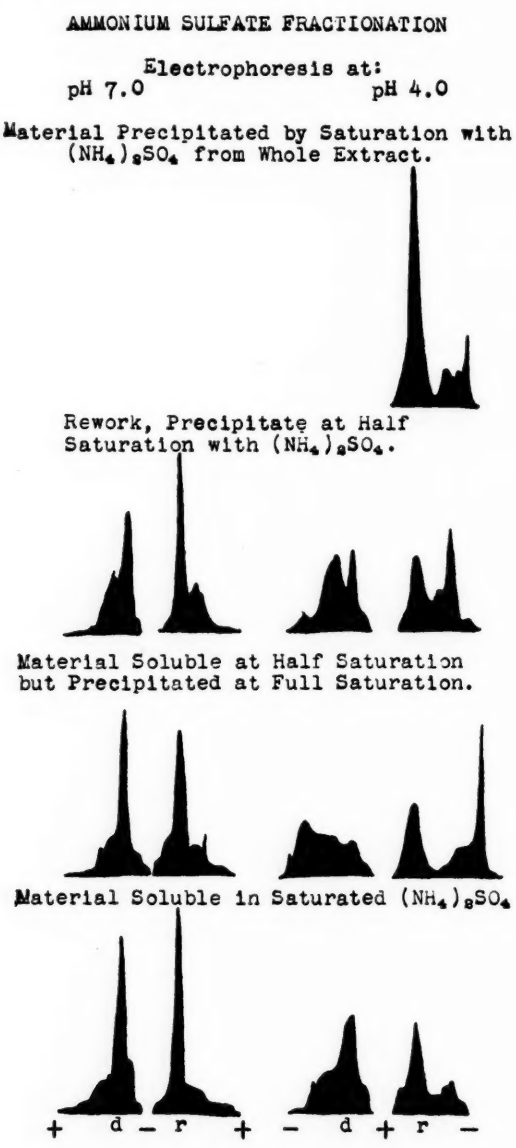
AMMONIUM SULFATE FRACTIONATION
Electrophoresis at:
pH 7.0
pH 4.0
Material Precipitated by Saturation with $(NH_4)_2SO_4$ from Whole Extract.
Rework, Precipitate at Half Saturation with $(NH_4)_2SO_4$.
Material Soluble at Half Saturation but Precipitated at Full Saturation.
Material Soluble in Saturated $(NH_4)_2SO_4$
+ d − r + − d + r −

Fig. 4.

based upon the amount of whole pollen from which it was derived. It was further fractionated by reprecipitating five times by dialysis, as before, against half-saturated ammonium sulfate at pH 4. The supernatants were dialyzed to full saturation, and the fraction precipitating

between half and full saturation was collected. Considerable colored organic matter remained in solution in the saturated ammonium sulfate solutions, so that all such were combined and dialyzed against tap water followed by distilled water, and then concentrated to a small bulk in front of a fan. The ammonium sulfate precipitates were taken up in distilled water and dialyzed sulfate free, as determined by testing the outer fluid, after standing, with barium chloride. All the fractions were eventually frozen and dried in vacuo.

The material insoluble in the acetate buffer at pH 4.0 and the material soluble in the saturated ammonium sulfate were practically inert by scratch test. The fractions precipitated at half saturation and at full saturation with ammonium sulfate at pH 4 were active, and both had approximately equal strength as determined by scratch test. The electrophoretic patterns are difficult to interpret (Fig. 4). The patterns at pH 7.0 show the prominent stationary peak in all fractions. The pH 4.0 insoluble material was too insoluble at pH 7.0 to give a satisfactory picture, and in all the other fractions the minor peaks having mobilities about 3.5×10^{-5} spread out so quickly that only slight differences in the patterns were observable. In the fraction precipitated at half saturation, and in the fraction soluble in saturated ammonium sulfate, there appears a suggestion of a small peak moving away from the others with a positive charge.

In agreement with the work of Stull[7] it was found that the fraction precipitated with half saturation of ammonium sulfate is practically carbohydrate-free since it gives only a faint Molisch reaction. In both alcohol and ammonium sulfate fractionations, the reaction was stronger in the fractions which were precipitated by the most concentrated solutions; hence the carbohydrate is one of the relatively more soluble constituents of the extracts.

At pH 4.0 there is a great deal of overlapping, but the fraction isolated between half and full saturation has a peak which has a mobility of 7.0×10^{-5} with a positive charge, and is faster than any peak present in the other two fractions. The stationary peak is very prominent in all fractions, and also a less definite peak having a mobility of about 3.8×10^{-5} is present. The precipitate at half saturation has small peaks with mobilities of 2.5 and 5.5×10^{-5} which are not definitely distinguishable in the others.

Nitrogen determinations were made on the dry powders obtained by lyophilizing the salt-free solutions from the alcohol and ammonium sulfate fractions. Since none of the fractions was a single substance, the results can show only trends, but when they are compared with the electrophoresis diagrams (where these are available), it is seen that the higher nitrogen values are associated with the presence of larger amounts of fast-moving components at both pH 4.0 and 7.0. Those which are low in nitrogen, however, such as the 60 to 80 per cent

TABLE I

FRACTION	ACTIVITY (SCRATCH TESTS)	PER CENT NITROGEN IN DRY MATERIAL	MOLISCH REACTION
Dialyzed and lyophilized unfractionated extract (Fig. 1)	++++	*	*
Phosphate fractions (Fig. 2)			
0 – 2 molar	++++	*	*
2 – 4 molar	+++	*	*
4 molar soluble	0	*	*
Alcohol fractions (Fig. 3)			
water – 15%	*	*	*
15% – 30%	+++	7.9	faint
30% – 60%	+++	7.9	slight
60% – 80%	+++	4.7	very strong
soluble in 80%	0	8.7	strong
Ammonium sulfate fractions (Fig. 4)			
whole extract precipitated at saturation	++++	*	*
pH 4 buffer insoluble	±	11.0	moderate
0 – ½ saturation	+++	10.4	faint
½ – full saturation	+++	11.4 0	moderate
soluble at full saturation	0	3.4	strong

*Not done.

alcohol fraction, and the material soluble in saturated ammonium sulfate, are relatively pure stationary components (Table I).

DISCUSSION

One of the purposes of this series of studies was to map out a method for isolating pure substances from ragweed pollen and to determine their chemical compositions and clinical values. In this regard, the studies have not been successful. They have, however, pointed out the complexity of the pollen extracts. Many workers have disputed this point and have offered various preparations as the pure active material; but from the results obtained here, the probability is that up to the present none of the fractions prepared by any chemical means even approaches the criterion of being a pure substance; this is shown by having a single peak in the electrophoretic pattern.

All the solutions examined in this work have been thoroughly dialyzed in Visking sacs so that the substances found are only those of a relatively large molecular size.

The nature of the stationary peak, which is so ubiquitous, needs further study. The most obvious explanation is that it is one of several substances having no net charge. There is a possibility that it is a complex substance. A large part of it appears to be very soluble in water since the peak becomes relatively larger in the fractions precipitated only by the higher concentrations of salts or alcohol; and its occurrence in the precipitates formed at lower concentrations may be

due to less soluble components in it, or to weak chemical combination with the other substances precipitated. It was found in the fraction precipitated by half-saturated ammonium sulfate which was free of carbohydrate; although, on the other hand, the material isolated by electrophoresis by Abramson, Moore and Gettner[4] gave a positive Molisch reaction. This peak does not appear to be merely a boundary anomaly such as the delta and epsilon boundaries seen in the examination of serum proteins,[6] for the latter are never the most prominent peaks, and, moreover, they differ in size.

The stationary peak is not directly connected with skin test activity since it occurs also in the inactive material soluble even in saturated ammonium sulfate at pH 4. The possibility that it had been denatured in this medium is not excluded, but it is obvious that the presence of the peak in a fraction is no indication of its activity.

SUMMARY

1. An electrophoretic study has been made of fractions separated from extracts of low ragweed pollen, prepared first by extraction with concentrated potassium phosphate buffers at pH 7.0; second, by extraction in the cold with ethyl alcohol of various strengths; and third, by precipitation of aqueous extracts by ammonium sulfate at one-half and at full saturation at pH 4.

2. All fractions, except those soluble in 80 per cent alcohol (by volume) or saturated ammonium sulfate, were active by scratch test on sensitive persons.

3. A peak in the electrophoretic patterns, which represents a substance or substances having no net charge at pH 4.0 or 7.0, was found in every fraction studied. This is the material which was described by Abramson, Moore and Gettner as "USD" or "artefolin." It is most prominent in the fractions low in nitrogen and high in carbohydrate. This peak cannot be used as a measure of the skin test-active substance nor is it related to carbohydrate.

4. Material extracted in 60 per cent alcohol at minus 15° C., and precipitated by 80 per cent alcohol was found to be the most nearly pure of all fractions, being composed largely of uncharged substance with two minor components. This fraction corresponds with Stull's substance "A."

5. All the fractions showed several components, but the electrophoretic patterns spread and overlapped to such an extent that it has not been possible to distinguish them clearly.

6. None of the different chemical means of fractionation of the pollen extracts have yielded any fraction which is even approximately a pure chemical substance.

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THE VOLUMETRIC INCIDENCE OF ATMOSPHERIC ALLERGENS

I. Specific Gravity of Pollen Grains

Oren C. Durham
Chicago, Ill.

SINCE the bacterial content of water and air has usually been recorded in terms of a volumetric unit, it has seemed logical that the incidence of air-borne allergens should likewise be stated on a volumetric basis. Thus, the cubic yard standard was early suggested, probably because the normal daily human intake of air is usually measured in cubic yards. Allergists have not as yet found a practicable method of direct volumetric census of air-borne allergens so there have been frequent attempts to convert the easily obtained gravity slide data to equivalent volumetric figures. For this purpose the following formula has been derived by Scheppegrell,[1] discussed by Cocke,[2] and revised by Dahl:[3]

$$n = \frac{6.97 \times N}{V \times t}$$

"where n is the approximate number of pollen grains per cubic yard of air, N the number of pollen grains per square centimeter of surface of the exposed slide, V the velocity of fall of the pollen grain in feet per second (derived from Stokes' law), and t is time (24 hours)."

Dahl expresses Stokes' law as follows:

$$V = \frac{2gr^2(d_1 - d_2)}{9K}$$

"where V represents velocity, g the acceleration of gravity (980 cm. per second), r the radius of the sphere (i.e. particle), d_1 and d_2 the densities of the sphere and medium, respectively, and K the coefficient of viscosity of the medium which, in this instance, may be taken to be 0.00018, the approximate coefficient of viscosity for air at temperatures of 20° to 23° C."

In applying Stokes' law, Scheppegrell omitted the density factor $(d_1 - d_2)$ without explanation. Cocke regarded this omission as "permissible" but later admitted that "a careful determination of the specific gravity* of the various pollens will doubtless shed much light on the question" of certain small discrepancies between his calculated and experimental rates of fall of various kinds of pollen grains. Recently Dahl has questioned "the propriety of omitting the density

From the Biological Laboratories of Abbott Laboratories.

*Both Cocke and Dahl have used the terms "density" and "specific gravity" to express the $\frac{\text{mass}}{\text{volume}}$ relationship of the whole, natural pollen particle, *not* the density of the organic substance of which it is composed. The terms are so used throughout this article.

factor from Stokes' formula'' and called attention to the present inadequate data concerning the specific gravity of pollen grains. This paper reports my attempts to weigh and measure pollen grains of the common hay fever types.

EXISTING DATA ON WEIGHT AND VOLUME OF POLLENS

Duke[4] and Thommen[5] are in virtual agreement on the weights of a cubic centimeter of dry, loose untreated pollen as shown below:

NAME	DIAMETER IN MICRONS	WEIGHT IN GM. PER C.C.	
		DUKE	THOMMEN
Common ragweed	16		0.32
Common ragweed	22	0.30	
Giant ragweed	18	0.30	0.34
Scotch pine	44		0.32
Scotch pine	45	0.32	
Austrian pine	52	0.28	0.34
Timothy	40	0.64	0.60
Corn	88		0.78
Corn	90	0.71	

The Duke figures published in 1925 include sixty-two other species besides the above. Thommen (1930) checked only six of the Duke list, including those with the greatest and smallest weights. Regardless of the gross volume of loose pollen involved in obtaining the above figures, it is obvious that a considerable portion of each measured cubic centimeter could be accounted for by empty space around the pollen grains. Even though the weighings may have been accurate, the volumes are not those of the net aggregate pollen grains, and any deductions from these figures regarding density can have only approximate and relative meaning. Nevertheless, as Dahl notes, the figures suggest appreciable differences in densities of pollen grains of different species.

In any critical study of the specific gravity of pollen grains, one of the first major difficulties is that caused by the hygroscopic nature of these particles. Beech pollen grains absorb water from the atmosphere so rapidly that a few dry granules on an open slide can be caused to expand merely by breathing on them. Fresh grass pollen grains shaken out of a ripened anther in their expanded form and allowed to dry on an open slide under observation, change from the opaque, spherical form to the clear, shrunken pattern in a very few moments. The change will take place very rapidly if the air over the slide is agitated.

Thus, both the weight and the volume, as well as the consequent density, of floating pollen grains of a given kind vary with the humidity of the air in which they happen to be suspended. This is emphasized (perhaps overemphasized) by the careful experiments of Buller[6] with mushroom spores. He found that spherical spores of *Amanitopsis vaginata* 10 microns in diameter fell three times as fast when fully expanded and falling in 100 per cent humid air, as when dry and shrunken in dry air.

ABSORPTION AND DESORPTION EXPERIMENTS (TABLE I)

In order to secure data on the reversible absorptive quality of common hay fever pollen grains experimental lots of commercially dried pollen were selected as follows:

		LOT
Ragweeds		
Common ragweed	*Ambrosia elatior*	A
Southern ragweed	*Ambrosia bidentata*	P
Cocklebur	*Xanthium commune*	B
Burweed marsh elder	*Iva xanthiifolia*	N
Amaranth-Chenopod		
Russian thistle	*Salsola pestifer*	G
Western water hemp	*Acnida tamariscina*	O
Grasses		
Corn	*Zea mays*	E
Timothy	*Phleum pratense*	D
Trees		
Oak	*Quercus imbricaria*	H
Black walnut	*Juglans nigra*	K
Alder	*Alnus glutinosa*	L
White ash	*Fraxinus americana*	M

TABLE I

DESICCATION AND ABSORPTION EXPERIMENTS WITH POLLEN
(WEIGHTS IN MILLIGRAMS)

KIND OF POLLEN	LOT	DESICCATION EXPERIMENTS			ABSORPTION EXPERIMENTS			MAXIMUM ABSORPTION	
		BOTTLE WEIGHT	DESICCATOR	ROOM	BOTTLE WEIGHT	HUMIDIFIER	ROOM	FLUCTUATION IN GRAMS	FLUCTUATION IN PER CENT
Common ragweed	A	203	196	200	200	329	198	136	70
Southern ragweed	P	200	199	202	200	293	202	94	47
Cocklebur	B	200	192	200	201	347	200	153	79
Russian thistle	G	201	195	200	203	324	203	125	64
Western water hemp	O	200	193	198	200	357	209	164	86
Corn	E	201	197	201	200	346	201	150	76
Timothy	D	212	201	222	205	399	201	200	100

The first effort was to determine how much water could be removed from commercially dried pollen. So 200 milligrams, more or less, was weighed, spread thin on a watch crystal and placed in a desiccator over calcium chloride. After several hours a small loss of weight was registered. The unit was then allowed to stand uncovered in the laboratory for a few hours and again weighed, at which time it was found to have absorbed almost exactly the same amount of moisture as it carried in the storage bottle. Another 200-milligram lot was subjected to extreme humidity by being placed in an airtight humidifier. After several hours in this chamber, a weighing showed rates of absorption over the desiccator weight varying with different species from 47 to 100 per

cent. This unit was also allowed to stand uncovered in the laboratory, and in a short time it returned to almost exactly its original bottle weight.

DENSITY EXPERIMENTS (TABLES II AND III)

In determining the approximate relative density of pollen grains, both direct and indirect methods were used. Table II gives the results of attempts to measure and calculate the volumes of pollen grains under the varying conditions which have been brought to view by the absorption experiments as reported above.

The micrometer measurements represent the mean diameters obtained by measuring a large number of granules in each lot of pollen. All measurements were carried out with a micrometer ocular under high power (magnification approximately 1400) using a cover glass and the oil immersion objective. Ragweed granules were measured both in oil (equivalent to dry) and in aqueous solution. In measuring ragweed granules the spicules were disregarded.

The calculated net volume figures in Table II were obtained directly from diameters by use of the formula for volume of a sphere ($4/3\ \pi\ r^3$). Since grass pollen granules in the dry state are not at all spherical, no calculations of volume were attempted except for expanded grains. An arbitrary mean diameter of 90 microns was used for corn, and 34 microns for timothy. These are, of course, only rough approximations. No calculations of volume were attempted for those tree and weed pollens which are not approximately spherical when dry.

The figures for "gross" experimental volume were obtained by placing several grams of pollen in a narrow graduated cylinder and tapping the bottom of the cylinder vigorously until the mass was packed to its minimum possible space. The "net" experimental figures were obtained by subtracting 26 per cent from the gross figure. This deduction represents the proportion of air space between nonelastic spheres of uniform diameter when packed together as tightly as possible.[7] Since dry pollen grains do not meet perfectly these standards of uniformity and rigidity, the resulting net figure is only approximately correct. Nevertheless, the results are probably as nearly right as those obtained by calculation from micrometer measurement, and are useful as a check on these calculations.

Even when expanded in water, ragweed granules show no appreciable increase in diameter. An idea of the approximate amount of expansion of moist ragweed pollen can be gained from the following experiment: two grams of short ragweed pollen were dried in the desiccator and then placed in a narrow graduated cylinder. Prolonged tapping of the bottom of the cylinder on the table packed the pollen to an apparent minimum gross volume of 5 c.c. The same lot was then spread out in a humidifier, and after twelve hours again tapped down in the graduated beaker. This time the apparent minimum gross vol-

TABLE II

DIAMETER AND VOLUME OF POLLEN GRAINS

KIND OF POLLEN	LOT	DIAMETER IN MICRONS*		CALCULATED NET VOLUME OF 1 GRANULE IN C.C. $\times 10^{-9}$			EXPERIMENTAL VOLUME OF 1 GRAM IN C.C. (DRY)	
		IN OIL	IN CALBERLA'S SOLUTION	VERY DRY	BOTTLE-ROOM	MOIST	GROSS	NET
Common ragweed	A	20	20	4.19	4.19	4.52	2.13	1.58
Southern ragweed	P	23	23	6.37	6.37	6.88	2.42	1.79
Cocklebur	B	27	27	10.30	10.30	11.12	2.55	1.88
Burweed marsh elder	N	15.5×22	19.25				1.73	1.28
Russian thistle	G	23.65	26	6.93	6.93	9.19	1.35	1.00
Western water hemp	O	24.75	27.5				1.35	1.00
Corn	E		84×99			381.81	1.20	.89
Timothy	D		35.7×30.4			20.65	1.35	1.00
Oak	H	22×35.75	30.25				1.3	.96
Walnut	K	38.5	35.75				1.45	1.07
Alder	L	26	25×21				1.39	1.03
Ash	M	19×32	28.6				1.5	1.11

*Equatorial, when only one measurement is shown. When two figures are given (except for grasses) the first is the equatorial diameter, the second polar.

TABLE III

WEIGHT AND SPECIFIC GRAVITY OF POLLEN GRAINS

KIND OF POLLEN	LOT	WEIGHT OF 1 POLLEN GRAIN IN GRAMS $\times 10^{-9}$			SPECIFIC GRAVITY				
					CALCULATED			EXPERIMENTAL (DRY)	PROBABLE OUTDOOR MEAN
		VERY DRY	BOTTLE-ROOM	MOIST	VERY DRY	BOTTLE-ROOM	MOIST		
Common ragweed	A	1.95	2.02	3.32	.47	.48	.73	.63	.55
Southern ragweed	P	3.05	3.07	3.50	.48	.48	.51	.56	.50
Cocklebur	B	3.76	3.92	6.80	.36	.38	.61	.52	.45
Burweed marsh elder	N							.79	
Russian thistle	G	5.12	5.29	8.40	.74	.76	1.09	1.00	.90
Western water hemp	O							1.00	
Corn	E	186.71	190.51	335.34			.88	1.10	1.00
Timothy	D	8.63	9.09	17.27			.84	1.00	.90
Oak	H							1.04	
Walnut	K							.93	
Alder	L							.97	
Ash	M							.90	

ume was 5.4 c.c. The increase in volume was therefore only about 8 per cent.

The weights shown in Table III were obtained by suspending a suitable amount of pollen direct from the storage bottle ("bottle" or "room" weight) in a convenient quantity of lactophenol solution* (for

*Phenol 20 per cent
Lactic Acid 20 per cent
Glycerin 40 per cent
Water 20 per cent

example, 50 mg. of pollen in 50 c.c. of solution) and counting in a Levy blood counting chamber. The weights under "very dry" were then calculated according to the loss of weight and drying as shown in Table I. The weights under "moist" were also calculated from data in Table I, but allowance was made for the 8 per cent expansion of the ragweeds as discussed above, and for Russian thistle as shown in the two different measurements in different media.

The calculated specific gravity figures are derived directly from the figures for calculated net volume of one granule and weight of one granule. The experimental figures for specific gravity were derived from the experimental volume of one gram as shown in Table II. The "probable mean" figures in the last column take into consideration both theoretical and experimental figures and the fact that free floating pollen in outdoor air must usually be relatively dry. It is interesting to note that the experimental specific gravity figures are in almost every case higher than those obtained by weighing and measuring single grains.

DISCUSSION

Approximate figures have been obtained for the specific gravity of commercially dried pollen granules of twelve species of common hay fever pollens. It was found that these pollens could be left exposed to varying conditions of humidity in the laboratory, even in warm, rainy weather, without appreciable change in weight. We may thus safely infer that the dryness of these bottled pollens does not differ essentially from that of free floating pollen grains in dry warm weather.

The ragweeds tested have densities of approximately 0.5, whereas that of the grass pollens tested approaches 1. The tree pollen grains average about 0.90 by the experimental method. From these results it will be seen that calculations already made by Scheppegrell, Cocke and Dahl, who have used Stokes' law for determining the rate of fall and number of pollens per cubic yard of air, may be expected to give reasonably accurate results for some tree and grass pollens, whereas they are evidently greatly in error for the ragweeds. For example, instead of a theoretical rate of fall of 0.04 foot per second, as previously calculated for short ragweed pollen of 20 microns diameter, the rate of fall, taking density into account, would be 0.022 foot per second. It follows that instead of 10 granules of ragweed pollen per square centimeter on a gravity slide denoting 73 pollen grains per cubic yard of air, the recalculated factor will give 132 pollen grains per cubic yard of air, or an increase of nearly 100 per cent over the previously accepted figure.

It must be remembered, however, that Stokes' law can be applied accurately only to bodies which are smooth and spherical. No species of pollen meets these requirements fully; many species are decidedly irregular both in shape and surface texture. So, before adopting new conversion formulas which will include only the density factors that

are brought to light by this study, it will be necessary to determine the actual rates of fall in still air of particular species of pollen. Experiments of this type will be detailed in a subsequent article.

Grateful acknowledgment is hereby made for helpful suggestions offered by Doctor R. M. Watrous and for the technical assistance of Mr. Ray E. Anderson.

SUMMARY

1. Adequate application of Stokes' law to the problem of volumetric pollen content of the air, even for fairly spherical pollen grains, requires reasonably accurate data on the specific gravity (density) of these granules.

2. The specific gravity (density) of free-floating pollen grains varies with their moisture content and with the consequent changes in volume and surface character of the affected grains.

3. While extreme moisture absorption may cause appreciable changes in the weight and volume of pollen grains, it is quite likely that free-floating pollen usually contains little, if any, more moisture than commercially dried pollen.

4. Approximate specific gravity figures are herewith submitted for typical weed, grass, and tree pollens, based on various experimental approaches.

5. The assumption that ragweed pollen grains have a specific gravity of 1 has led to the publication of calculated factors for rate of fall and volumetric ragweed air content which are in error nearly 100 per cent, except as that error may be exceeded or diminished under actual outdoor conditions by factors which have as yet not been recognized or evaluated.

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THE EFFECT OF SODIUM, POTASSIUM, AND THIOSULFATE IONS ON ANAPHYLAXIS

ROBERT G. CARLSON, M.D., AND RICHARD W. WHITEHEAD, M.D.
DENVER, COLO.

WITHIN recent years, much interest has developed concerning the use of electrolytes and the effect of water balance in the treatment of certain allergic conditions.

As early as 1921, August Lumière recommended the use of sodium thiosulfate in the treatment of allergic conditions. In his treatise,[1] he states that since tissues of living beings are composed in large part of colloids, their reactions are dependent on the state of these colloids, and should obey the laws governing their action. Lumière correlated the state of the cellular colloids with disease and pathologic conditions. He believed that flocculation of the cell membrane colloids changed normal reactions into abnormal states. Particularly pertinent was the fact that the exchange between cell and body fluids was altered. Thus, various foreign substances injected parenterally or absorbed from the gastrointestinal tract tend to change the normal reactivity of the cell by alteration of the colloidal state. Lumière, therefore, hoped that certain therapeutic agents could be found which would disperse a flocculation produced by an untoward reaction. He found that sodium thiosulfate added to flocculates in test tube experiments caused a dispersion of the particles. Thus, he concluded that here was an agent which might correct the colloidal state which he believed determined disease, particularly that of an allergic type.

This hypothesis seemed to lie dormant until fairly recently when, in 1935, Blina,[2] in Italy, engaged in experimental work on the problem. He found that in sensitive guinea pigs, sodium thiosulfate was ineffective if administered following the shock dose. He observed also that the salt did not prevent sensitization if given immediately or within two hours following the sensitizing injection. He did find, however, that if the sodium thiosulfate was mixed with the antigen in ratios of 1:6 and 3:8, it prevented shock symptoms in sensitive pigs.

In 1930, Boissel[3] reported experimental work in which he demonstrated the effectiveness of the thiosulfate salt of magnesium. It is difficult to correlate this work with the present research, since he used the magnesium salt. He emphasizes, however, the effect of the thiosulfate radical in his results. Lumière and Monchal[4] (1937), also working with the magnesium salt, considered that rise in the cholesterol content of the blood was the factor responsible for the favorable therapeutic effects.

From the Department of Physiology and Pharmacology, University of Colorado School of Medicine.

Supported in part by a grant from the Therapeutic Research Committee of the American Medical Association.

Wendt[5] (1937) reviewed the therapeutic uses of sodium thiosulfate. Among the antiallergic applications were three cases of dermatitis which responded to intravenous administration of sodium thiosulfate. Very little was said as to the mechanism of the therapy in allergy, but it was stated that the sulfhydril groups might act upon the colloidal setup of the body fluids and cell membranes.

Early in 1938, Klein[6] reiterated Lumière's theories as to the pharmacology of sodium thiosulfate. He cited six cases, including migraine, urticaria, and an Arthus reaction, which responded remarkably well to sodium thiosulfate. He expressed the firm belief that the salt effects a colloidal change.

The influence of electrolyte changes in body fluids upon anaphylactic reactions has also received much attention from investigators in recent years. In particular, the similarity of the vascular responses to epinephrine and to potassium has stimulated workers to determine the effect of the latter in anaphylaxis and allergy.

Camp and Higgins[7] (1936) found that the injection of epinephrine produced an increase in serum potassium. A comparison of vascular effects produced by the injection of epinephrine with those produced by the injection of potassium led these workers to conclude that the action of epinephrine is due to a potassium shift into the circulation. D'Silva,[8, 9] in 1934 and 1936, also found that there was a transitory increase in serum potassium following the injection of epinephrine, and that it was due to the mobilization of potassium from the liver.

In 1929, Unshelm[10] reported no constant shift in serum potassium values in asthmatic patients. His potassium concentrations ran between 19.4 mg. and 22 mg. per cent. Kuschnarjew[11] (1930) found only a slight increase in serum potassium after anaphylactic shock in guinea pigs. Administration of potassium chloride and of potassium iodide did not prevent shock in his experiments. Wenner and Buhrmester[12] (1937) reported marked differences in potassium concentrations in normal, sensitized, and shocked guinea pigs. The average levels were respectively 26 mg., 33 mg., and 44 mg. per cent.

In 1938, Rusk and Kenamore[13] reported the beneficial use of potassium chloride, 4 to 6 Gm. daily, added to a low sodium acid-ash diet in five cases of urticaria. Cohen,[14] later in the same year, attempted to repeat the work of Rusk and Kenamore. Of eight cases treated, none showed improvement, and two cases manifested increased symptoms.

Stoesser and Cook[15] (1938) reported that potassium chloride, added to a low sodium diet in children, exerted a profoundly favorable effect upon the course of asthmatic symptoms. The additional use of pitressin further altered the potassium and sodium balance of the body with evident good results.

In 1938, Bloom[16] reported the relief of symptoms in hay fever and asthma in twenty-nine cases with the use of 1 Gm. daily of potassium

chloride. In 1939 he[17] presented the results of potassium chloride therapy in a number of types of allergic disorders. The majority of sixty-one cases of hay fever were definitely benefited by doses of potassium chloride up to 10 grains five times a day. Chronic types of allergic conditions did not respond well to the therapy.

Rusk, Weichselbaum and Somogyi,[18] in 1939, found that the concentration of serum potassium in allergic individuals was definitely higher than that in normal individuals. They administered insulin as a means of redistributing the potassium, thereby increasing the intracellular content at the expense of the serum concentration. The same result was obtained by the ingestion of glucose. In some cases in which both potassium chloride and insulin were used, good results were obtained.

In 1939, Abt[19] reported marked improvement in twenty cases of allergic children by the use of 1 to 5 grains of potassium chloride dissolved in water, three times a day.

Smith and Steffensen[20] (1940), in a series of 117 patients, obtained improvement or complete recovery by the administration of 10 grains of potassium chloride in a glass of water, three times a day. Parker[21] (1940) studied the potassium levels in twenty-eight normal and thirty-five allergic individuals. He found a characteristic potassium level for each individual. Oral administration of potassium chloride or potassium gluconate gave relief in most of the allergic individuals. The potassium level of the blood showed the least rise in those experiencing the greatest benefit from the therapy.

There have been a number of recent reports[22-27] (1939 and 1940), which showed chiefly negative results with potassium chloride therapy in allergic conditions. The number of cases unimproved by this treatment were 339 of a total of 416.

In 1940, Stoesser and Cook[28] reported extensive studies on twelve children and young adults with intractable asthma. These patients were hospitalized and given a low sodium diet, with improvement among some. Further loss of sodium was achieved by hyperthermia, which further improved the condition in most of the cases. The administration of sodium chloride produced an immediate increase in asthmatic symptoms. The addition of 6 to 10 Gm. of potassium daily to the low sodium diet terminated the milder attacks of asthma. The more severe attacks failed to respond to the administration of the potassium chloride, but did cease upon the withdrawal of this salt.

The effects of sodium administration, sodium loss, and potassium administration were studied in guinea pigs by Rubin and Rapoport[29] (1939). Depletion of body sodium, and potassium chloride injections definitely protected the animals from the shock dose of antigen.

The purpose of the present investigation is to show the relative effect of sodium thiosulfate, potassium chloride, and potassum thiosulfate upon anaphylaxis in guinea pigs.

METHODS AND RESULTS

Guinea pigs ranging in weight from 250 to 500 grams were sensitized by a subcutaneous injection of 0.2 c.c. of sheep serum. All animals received the shock dose of serum two to four weeks following the sensitizing injection. In each series of experiments the animals were evenly divided as to sex.

A. Determination of Minimum Lethal Dose of Serum.—An initial group of eleven animals was used to determine the minimum lethal dose of serum. With each subsequent experimental group, the M.L.D. was tested on a few animals to verify the original finding. The M.L.D. as a 1:10 dilution of the sheep serum in normal saline was administered by intravenous injection into the marginal ear vein.

Table I shows that 0.2 c.c., 1:10, of the sheep serum is the critical M.L.D.

TABLE I

SHOCK DOSE (1:10)	0.12 C.C.	0.15 C.C.	0.20 C.C.	0.25 C.C.
Recovered	3	3	7	0
Died	1	3	27	3

The effects of the salts were tested on the animals by two methods; in one, the antigen was mixed with the salt; in the other method, the salt was injected intraperitoneally or intravenously a short interval before the shock dose of serum was given.

B. Anaphylaxis as Affected by Previous Injections of Sodium Thiosulfate.—In this series seven guinea pigs received 4.0 c.c. of sodium thiosulfate, 1:10, intravenously. The pigs were given the shock dose after an interval of one-half to four hours. Four animals died following the M.L.D. of 0.2 c.c., 1:10, of sheep serum. A reduction in the shock dose to 0.1 c.c., 1:10, resulted in deaths of three animals. Thus, the sodium thiosulfate afforded no protection and probably produced some increase in sensitivity.

TABLE II

GUINEA PIG	MIXTURE	TIME MIXTURE STOOD BEFORE INJECTION	RESULT
171	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	1½ hr.	Died
178	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	1½ hr.	Died
179	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	3 hr.	Died
180	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	3 hr.	Died
181	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	4 hr.	Died
170	0.1 c.c. of (1.0 c.c. ss + 6 c.c. $Na_2S_2O_3$ 1:5)	6 days	Died
174	0.1 c.c., 1:10, ss + 1 c.c. $Na_2S_2O_3$ 1:5	--	Died
177	0.1 c.c., 1:10, ss + 1 c.c. $Na_2S_2O_3$ 1:5	--	Died

SS = Sheep serum.

C. Anaphylaxis as Affected by Mixtures of Sodium Thiosulfate and Serum.—This series was carried out in an attempt to substantiate the work of Blina. A simple mixture of sodium thiosulfate and serum

was kept for varying periods of time before injection into the animals. Table II indicates the composition of the mixtures, the time the mixtures were allowed to stand before injection, and the amount injected. The amount of the mixture injected (0.1 c.c.) corresponds to 0.14 c.c., 1:10, of the sheep serum. All animals died in shock with doses less than the M.L.D. of serum.

D. The Effect of Potassium Chloride Mixed With the Antigen.—It was found that an intravenous dose of 0.4 to 0.6 c.c. of a 5 per cent solution of potassium chloride was well tolerated by the guinea pigs. This amount was mixed with a 1:10 dilution of the sheep serum in physiologic saline and injected intravenously. Table III indicates the results obtained. It may be seen that by this method of administration, potassium chloride affords some protection. There is a statistically significant difference between these results and those obtained in the controls.

TABLE III

SHOCK DOSE (1:10)	0.12 C.C.	0.15 C.C.	0.2 C.C.	0.3 C.C.
Recovered	2	7	3	1
Died	0	1	5	0

E. Potassium Chloride Injected Intraperitoneally, Followed by the Antigen Intravenously.—The potassium chloride solution, 5 per cent, was injected intraperitoneally. After ten to twenty-five minutes, the shock dose of antigen in a 1:10 dilution was injected intravenously.

TABLE IV

GUINEA PIG	KCl (5%)	INTERVAL	SHOCK DOSE (1:10)	REACTION
287	3.0 c.c.	0	0.15 c.c.	Mild
284	3.0 c.c.	0	0.18 c.c.	Moderate
230	5.0 c.c.	11 min.	0.2 c.c.	Mild
231	5.0 c.c.	20 min.	0.2 c.c.	Died
232	4.0 c.c.	21 min.	0.2 c.c.	Mild
234	6.0 c.c.	20 min.	0.2 c.c.	Mild
233	6.0 c.c.	14 min.	0.2 c.c.	Mild
235	6.0 c.c.	25 min.	0.2 c.c.	Died
282	5.0 c.c.	22 min.	0.2 c.c.	Moderate
285	4.0 c.c.	20 min.	0.2 c.c.	Moderate
286	5.0 c.c.	20 min.	0.2 c.c.	Mild

As may be observed from Table IV, seven animals recovered, and two died following the M.L.D. of 0.2 c.c. sheep serum (1:10).

F. Potassium Thiosulfate Mixed With the Antigen.—A 6.3 per cent solution of potassium thiosulfate mixed with a 1:10 dilution of the sheep serum was injected intravenously. The potassium ion concentration in this solution is equal to that in the 5 per cent KCl solution. Intravenous injections of 0.6 to 0.8 c.c. of potassium thiosulfate, mixed with the M.L.D. of 0.2 c.c. of sheep serum (1:10) were administered to twelve animals. Seven animals recovered and five died as a result of this procedure.

G. Potassium Thiosulfate Injected Intraperitoneally.—This salt was injected intraperitoneally in concentrations of 6.3 and 3.15 per cent. After an interval of 12 to 40 minutes, the antigen was injected intravenously. Table V gives the pertinent facts of this experiment. Of nine animals treated, four recovered from the M.L.D. of serum.

TABLE V

GUINEA PIG	$K_2S_2O_3$ DOSE	INTERVAL TIME	SHOCK DOSE (1:10)	REACTION
315	2.0 c.c., 3.15%	28 min.	0.18 c.c.	Died
313	4.0 c.c., 3.15%	20 min.	0.2 c.c.	Died
314	2.0 c.c., 3.15%	23 min.	0.2 c.c.	Died
316	4.0 c.c., 6.3%	29 min.	0.2 c.c.	Died
317	2.0 c.c., 6.3%	30 min.	0.2 c.c.	Severe
318	2.0 c.c., 6.3%	33 min.	0.2 c.c.	Moderate
319	2.0 c.c., 6.3%	40 min.	0.2 c.c.	Mild
345	2.0 c.c., 6.3%	12 min.	0.2 c.c.	Severe
346	3.0 c.c., 6.3%	20 min.	0.2 c.c.	Died

DISCUSSION

The data from our investigation on guinea pigs reveal no such beneficial results from the administration of sodium thiosulfate as are recorded in the literature. From both clinical and experimental observations, it appears that the sodium ion is a contributing factor in allergic manifestations. Although our results indicate an increase in the anaphylactic reaction following the administration of the sodium salt, the degree of increase is not significant since $X^2 = 1.2$ in each series in which the sodium ion was employed.

Potassium therapy, however, produces definitely beneficial results irrespective of the mode of administration. Since there are no appreciable differences in results between the use of the chloride or of the thiosulfate salt of potassium, the thiosulfate ion appears to be of no therapeutic value in allergic conditions.

Conservatism in conclusions must be held, in view of the small number of animals used in each series, and the restricted conditions of the experiments. The limited range of dosage imposed by the M.L.D. technique does not permit the determination of the degree and range of effectiveness of the potassium ion. Within the small range of lethal antigen dosage, the potassium ion obviously has a beneficial effect.

SUMMARY

1. The effects in anaphylaxis of sodium, potassium, and thiosulfate ions have been studied by the minimum lethal dose technique.

2. Sodium thiosulfate shows no inhibitory effect on the anaphylactic reaction.

3. Potassium salts are definitely of value in the prevention of death from the M.L.D. of the antigen.

4. No difference was noted between the effects of the chloride and thiosulfate salts of potassium.

5. The method of intraperitoneal injection of the salts a short interval before the shock dose showed no significant advantage over the method of simultaneous administration of the salts with the antigen.

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THE WELTMANN REACTION IN BRONCHIAL ASTHMA

SUSAN C. DEES, M.D.
DURHAM, N. C.

WITH THE TECHNICAL ASSISTANCE OF SUSAN SPELL

THE Weltmann reaction has been used as a laboratory test for detecting the presence of infection in a wide variety of clinical conditions.[1] Its use in bronchial asthma was suggested in a previous paper.[2] The present study summarizes the results of the Weltmann reaction in 224 cases of bronchial asthma. The technique for performing the test is briefly as follows:

Solutions ranging from 0.1 to 0.01 per cent are prepared from a stock solution of 10 per cent $CaCl_2.6H_2O$ (Merck). Two and one-half cubic centimeters of each of these solutions are measured into small test tubes which are numbered from 1 to 10 to correspond with the dilutions, beginning with 0.1 per cent solution as Tube 1. To each tube is added exactly 0.05 c.c. of unhemolyzed serum. The contents of each tube is thoroughly mixed, and the tubes in order, are placed in a rack in a boiling water bath for fifteen minutes. They are then removed, and the number of tubes in which coagulation is present is noted. Normal serum is regularly coagulated in the first six tubes in concentrations ranging from 0.1 per cent through 0.05 per cent $CaCl_2.6H_2O$. The values are expressed as a coagulation band corresponding to the number of tubes showing coagulation.[3]

The Weltmann reaction on serum from normal adults and children has a remarkably constant value, a coagulation band of 6. Acute infections and inflammatory processes have coagulation bands of less than 6, while those conditions characterized pathologically by the formation of fibrous tissue have coagulation bands greater than 6. Should acute inflammation and healing, or fibrosis occur simultaneously, the Weltmann reaction will reflect the balance between the two processes. In such a state, a band of 6 may result, or the band may be shifted slightly to values higher or lower than 6.

In many instances, the Weltmann reaction has apparently been the laboratory test which most accurately followed the clinical course of a patient at any given moment. This has been true particularly in patients with progressive infections.[4] It has been observed that serum from allergic individuals has a coagulation band of 6. In the study of allergic disorders previously mentioned,[2] it was shown that infection influenced the coagulation band in exactly the same fashion in allergic, as in nonallergic patients. In spite of uncertainty as to the mechanism of the reaction, empirical studies of the test in many different diseases

From the Departments of Pediatrics and Medicine, Duke University School of Medicine and Duke Hospital, Durham, N. C.

have been done. In general, there is no definite value for the test in any given disease, with the exception of lobar pneumonia and nephrosis which have bands of zero, and Laënnec's cirrhosis which has bands of 9 or 10. The various types of diseases fit into a scheme as shown in Table I.

TABLE I

SCHEME OF COAGULATION BANDS

1-5	6	7-10
Inflammation Infection Necrosis	Normal and allergic conditions	Fibrotic and degenerative processes

In order to make an objective evaluation of the sensitivity of the Weltmann reaction as an index of infection in allergic patients, the present series of 224 asthmatics (51 children and 173 adults) were studied. In this group of patients, the clinical diagnosis was assumed to be correct and was used as the sole criterion for differentiating the cases into those without, and those with, varying degrees of infection. No attempt was made to correlate the Weltmann reaction with clinical impression until after the final diagnosis had been made by independent observers on the basis of clinical observation and examination, together with laboratory procedures exclusive of the Weltmann reaction. Those individuals with definite or suspected infection were examined exhaustively to determine its extent. The focus of infection was most frequently in the respiratory tract, so that nose and throat consultations were obtained in practically every case. The diagnosis and classification of the infection by the otolaryngologist was accepted as the most accurate clinical evaluation of the infection.

In addition to special study of the nasopharynx, all patients had a complete medical and allergic investigation. This included history, physical examination, chest x-rays, sinus x-rays, and intradermal skin tests to 110 common allergens. Complete blood studies with hemoglobin, white blood count, differential and sedimentation rate, as well as Weltmann reaction were done. Cultures from the nose and throat or sputum were obtained from almost all patients. In most cases a mixture of anaerobic organisms as well as the common aerobic flora, namely alpha and beta hemolytic streptococci, *Micrococcus pharyngis siccus, Micrococcus catarrhalis,* and staphylococci were present. A vaccine was made in the usual fashion from each organism isolated. Intradermal skin tests using not more than 0.05 c.c. of 1:10 dilution, and of the undiluted vaccine were made for all the organisms isolated from any patient. The skin reactions were read within thirty minutes and in twenty-four hours. These reactions were interpreted as follows: erythema alone doubtful, (plus-minus); wheal less than 0.5 cm. in diameter, 1 plus; wheal 0.5 cm. to 1 cm. in diameter, 2 plus; wheal greater than 1 cm. in diameter, 3 plus; wheal with pseudopods greater

than 2 cm. in diameter, 4 plus. For brevity, in Tables III and IV the positive skin reactions are indicated only as minus, plus-minus, and plus. The skin reactions to bacterial vaccines were used to corroborate the presence of demonstrable clinical infection, but were in no instance taken as the sole basis for diagnosing the presence of infection.

In every case, intradermal skin tests to the common allergens were done. Nearly all of the patients had positive skin reactions to one or more of the allergens such as pollens, inhalants, or foods. The same scale was used in the interpretation of those reactions as for the bacterial vaccines. The patients with asthma alone, or with other allergy, chiefly allergic rhinitis, had the highest number of positive skin reactions to allergens, and the fewest positive skin reactions to bacteria. The group with chronic infections had 75 per cent positive skin reactions to their autogenous vaccines. One-half the asthmatics with acute infections were skin sensitive to bacterial vaccines.

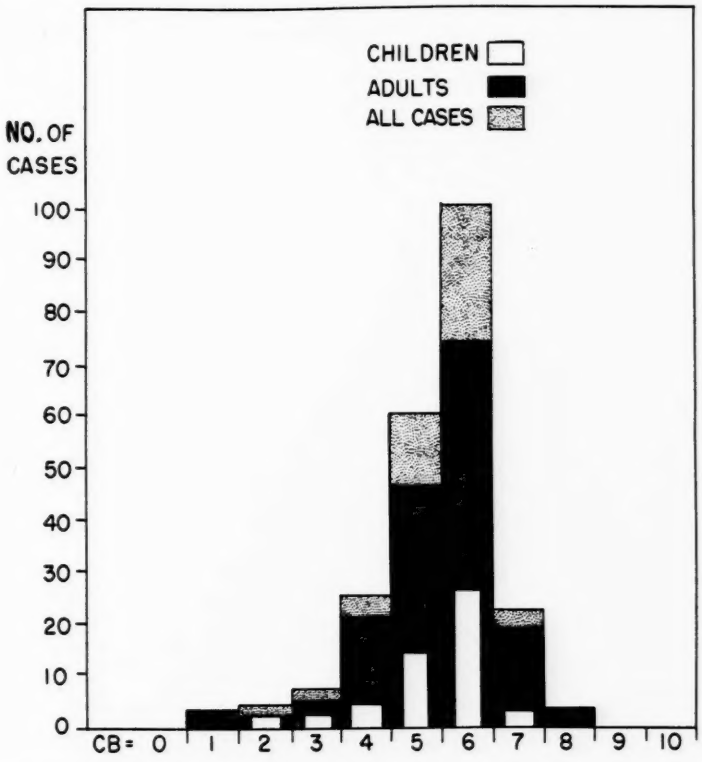

Fig. 1.—Distribution of 224 cases of bronchial asthma with respect to the length of the coagulation band.

Fig. 1 shows the distribution of patients with respect to the length of the coagulation band. One hundred, or 44.5 per cent of the entire group, had coagulation bands of 6. This number includes twenty-six, or 50 per cent of the children in the entire group.

To compare clinical findings with the coagulation band the patients have been separated, on the basis of clinical diagnosis, into five groups

as shown in Table II: (1) Uncomplicated asthma, (2) asthma associated with other allergic conditions, (3) asthma with complications of a noninfectious character, (4) asthma accompanied by chronic infections, and (5) asthma complicated with acute infection.

TABLE II

CLASSIFICATION OF 224 ASTHMATICS WITH RESPECT TO COAGULATION BAND

	CB = 1	CB = 2	CB = 3	CB = 4	CB = 5	CB = 6	CB = 7	CB = 8	CB = 9	CB = 10
Asthma, uncomplicated					6	30				
Asthma, accompanied by other allergic conditions						37				
Asthma, with complications of noninfectious character (pulmonary fibrosis, emphysema, heart disease, anemia, etc.)				1*		9	15	3		
Asthma, with chronic infections				9	40	24	7			
Asthma, with acute infections	3	4	7	15	14					
Total cases	3	4	7	25	60	100	22	3		

*Osteogenic sarcoma.

All of the patients with uncomplicated asthma due to extrinsic factors had coagulation bands of 6. A small group of six patients, who were diagnosed by the clinicians as having intrinsic or bacterial allergy, form an exception, as they all had bands of 5. From analysis of the histories in these patients, we believe that they had some active intrapulmonary infection, as well as sensitivity to bacterial allergens, but since we have not taken any liberties with reinterpreting the final clinical diagnosis, and no focus of infection was specifically diagnosed, these patients have been included among the uncomplicated asthma group. The evidence that intrapulmonary infection was present in all six patients consists of the following findings: all patients had considerable purulent sputum, chronic cough, and dyspnea. Four of them had persistent low-grade fever. They were all highly skin sensitive to their autogenous vaccines, and showed completely negative skin reactions to the common allergens. There was no demonstrable focus of infection outside the chest. Chest x-rays showed more increase in peribronchial markings in these patients than in any others of the entire series.

The second group, consisting of thirty-seven patients with asthma, associated with some other allergic disorder, all had coagulation bands of 6.

The third group of asthmatics, with complications of a noninfectious nature, had coagulation bands ranging from 4 to 8. All except one

patient in this group had bands of 6 or longer. The one band of 4 was found in a 15-year-old girl, a known asthmatic for five years, who also had an osteogenic sarcoma of the tibia of six months' duration. This girl was receiving x-ray therapy at the time the Weltmann reaction was done. It is known that malignant tumors, particularly of the bone, have a tendency to shorten the coagulation band.

In this group (Group 3) either emphysema, pulmonary fibrosis, arteriosclerotic and hypertensive cardiovascular disease, pernicious anemia, polycythemia, cirrhosis of the liver, or syphilis were complicating factors. Emphysema and pulmonary fibrosis were present in nearly every patient, the mild cases generally having bands of 6. The more marked ones associated with clubbing of fingers or gross chest deformity had bands of 7 or 8.

One-third of the entire series, or eighty patients, comprising Group 4, had clinical evidence of chronic infection, chiefly in the upper respiratory tract. This included tonsillitis, sinusitis, bronchitis, bronchiectasis, either singly or in combination. The coagulation bands here ranged from 4 to 7. There were only seven patients with bands of 7, and, in addition to chronic infection, all had some noninfectious process such as emphysema, pulmonary fibrosis, or heart disease, as additional findings. Twenty-four asthmatics with coagulation bands of 6 were found to have some chronic infection. Since this group of twenty-four patients illustrates the fact that the Weltmann reaction can be an expression of the balance between two different pathologic processes, these cases are summarized in detail in Table III. Approximately one-half of these patients had very slight infection, principally chronic tonsillitis and adenoiditis, sinusitis, or bronchitis. The other half had more severe chronic or multiple infections which were offset by clinical evidence of conditions tending to produce a long coagulation band, usually extensive pulmonary fibrosis. The infections found in this portion of the group are bronchitis, purulent sinusitis, and tuberculosis. The asthmatics with chronic infections who had coagulation bands of 4 or 5 are differentiated from each other, and from those with coagulation bands of 6, only by the fact that they have extensive or multiple foci of infection without other complicating conditions of a fibrotic nature. They are also considerably younger as a group than those with bands of 6, their average age being less than 35 to 40 years, while those with coagulation bands of 6 are older than 40 years.

The fifth clinical group, consisting of forty patients, all had evidence of acute infection in addition to bronchial asthma. The coagulation bands ranged from 1 to 5. The infections noted were acute tonsillitis, sinusitis, bronchitis, capillary bronchitis, and bronchopneumonia. This was the youngest age group, as most of the adults were less than 30 years old, and almost all the children with infections fell in this group.

An analysis of these asthmatics should include a comparison of the Weltmann reaction and other established laboratory tests, as well as

TABLE III

24 ASTHMATICS WITH CB = 6, COMPLICATED WITH CHRONIC INFECTIONS

CASE	AGE YR.	CLINICAL DIAGNOSIS	CB	W.B.C.	STABS	E.	C.S.R.	SKIN TESTS	
								ALL.	A.V.
Mild Chronic Infections									
A. C.	33	Chronic tonsillitis and adenoiditis	6	8,000	2	10	18	+	−
J. W.		Chronic tonsillitis and adenoiditis	6	7,100	1	3	5	+	−
V. R.	26	Chronic tonsillitis and adenoiditis	6	5,700	3	0	16	+	+
A. B.	37	Chronic tonsillitis and adenoiditis	6	8,700	2	10	23	+	−
R. W.		Chronic bronchitis	6	7,350	1	3	19	+	−
I. H.	19	Chronic tonsillitis and adenoiditis, sinusitis, bronchitis	6	6,050	0	10	15	+	+
J. C.	30	Chronic tonsillitis and adenoiditis, sinusitis	6	9,800	3	2	4	+	+
B. E.	30	Allergic rhinitis, chronic tonsillitis, and adenoiditis	6	8,350	0	10		+	−
C. D.		Allergic rhinitis, chronic tonsillitis, and adenoiditis	6	12,450	4	5	8	+	−
A. C.	15	Allergic rhinitis, chronic sinusitis	6	6,700	0	13	9	+	−
M. C.	34	Allergic rhinitis, chronic sinusitis	6	8,500	1	2	19	+	−
Chronic Infection and Fibrosis									
W. H.	18	Allergic rhinitis, bronchitis, pulmonary fibrosis	6	15 600	0	3	38	+	−
W. C.	30	Arrested pulmonary tuberculosis with extensive pulmonary fibrosis, partial pneumothorax	6	6,300	0	1	26	+	+
N. S.	26	Questionable pulmonary tuberculosis, chronic sinusitis	6	9,600		4	31	+	+
E. D.	59	Allergic rhinitis, arrested fibrotic, pulmonary tuberculosis, generalized arteriosclerosis	6	14,550	0	5	15	+	−
A. S.	28	Allergic rhinitis, chronic tonsillitis and adenoiditis, bronchitis, rheumatoid arthritis, inactive	6	6,400	0	13		+	+
L. K.	44	Allergic rhinitis, chronic purulent sinusitis, pulmonary fibrosis	6	15,100	0	25	22	±	±
M. M.	33	Allergic rhinitis, chronic bronchitis, emphysema	6	11,050	1	4	44	+	+
C. B.	62	Chronic tonsillitis, adenoiditis, bronchitis, pulmonary osteo-arthropathy	6	14,000	3	1	25	+	+
A. C.	59	Pulmonary fibrosis, bronchiectasis hyperthyroidism	6	15,360		5	15	+	−
A. L.	68	Chronic bronchitis, emphysema, generalized arteriosclerosis, cor pulmonale	6	9,250	2	4	15	+	−
J. B.	49	Chronic purulent sinusitis. emphysema, generalized arteriosclerosis	6	9,700	4	1	19	±	+
A. H.	47	Chronic sinusitis, pulmonary fibrosis	6	13,200	4	12			
C. P.	57	Chronic purulent sinusitis	6	8.150	6	13			

Key: CB = Coagulation band
W.B.C. = White blood count
E. = Eosinophiles
C.S.R. = Corrected sedimentation rate
All. = Allergens
A.V. = Autogenous vaccine

skin tests. It is impossible to present each case of the series in detail. Table III shows this data for the patients with complications whose coagulation band is 6. The remainder of the cases have been summarized in Table IV, again using the clinical diagnosis as the basis for classification. The Weltmann reaction, white count, sedimentation rate, and results of skin tests are shown here.

TABLE IV

SUMMARY OF CLINICAL CONDITION, BLOOD AND SKIN TESTS IN 224 ASTHMATICS

NUMBER OF CASES	ADULTS	CHILDREN	CB	W.B.C.	AVERAGE W.B.C.	CORRECTED SED. RATE	SKIN TESTS ALL.	SKIN TESTS A.V.
I. Uncomplicated Asthma								
30	23	7	6	3,800-15,160	7,900	0-43 mm./1 hr.	24+ 6–	14+ 16–
Intrinsic asthma 6	6		5	5,300- 9,550	8,200	10-45 mm./1 hr.	4+ 2–	6+
II. Asthma and Other Allergy (Allergic Rhinitis)								
37	18	19	6	5,200- 9,750	8,000	3-43 mm./1 hr.	18+	5+ 13–
III. Asthma and Noninfectious Complications								
28	1		4	3,000	3,000	30 mm./1 hr.	1+	1+
	9		6	4,520-17,400	8,500	5-32 mm./1 hr.	4+ 5–	4+ 4– 1+
	12	3	7	4,750-18,160	9,750	1-19 mm./1 hr.	10+ 3– 2±	13+ 2–
	3		8	5,350- 8,850	6 800	0- 9 mm./1 hr.	3+	3+
IV. Asthma and Chronic Infection								
80	9		4	6,800-12,800	8,600	9-42 mm./1 hr.	8+ 1–	8+ 1–
	33	7	5	5,500-17,000	10,000	3-43 mm./1 hr.	33+ 6–	34+ 4–
	24		6	9,000-15,000	9,900	4-44 mm./1 hr.	20+ 2– 2±	13+ 9+ 2±
	7		7	6,700-12,000	8,650	5- 9 mm./1 hr.	6+ –	5+ 2–
V. Asthma With Acute Infection								
43	3		1	6,300-14,000	11,400	44 mm./1 hr.	3+	2+ 1–
	2	2	2	11,560-15,950	12,750	20-30 mm./1 hr.	3+	4+
	5	2	3	9,200-33,000	15,300	2-48 mm./1 hr.	1– 7+	6+ 1–
	11	4	4	6,700-14,400	9,800	9-44 mm./1 hr.	14+ 1–	13+ 2–
	7	7	5	3,950-20,000	9,600	7-40 mm./1 hr.	13+ 1–	10+ 4–

Key: See footnote, Table III.

In general, the white counts and sedimentation rates are lower in patients without infection than in those with infection. Many of the patients had unusually slow sedimentation rates, a finding frequently mentioned as characteristic of allergic individuals. There is no close

correlation between white count and sedimentation rate, or between either of these tests and the Weltmann reaction. There was no striking variation in the eosinophilia in the five groups of asthmatics. The average for all asthmatics was 7.4 per cent.

To recapitulate, the patients with allergy alone had coagulation bands of 6. Those with infections had bands less than 6, depending on the extent of the infection. Those with other noninfectious diseases had bands longer than 6. When infections and pulmonary fibrosis, for example, occurred together, the resulting coagulation band was determined by the balance between the two processes. When the Weltmann reaction is 6 or greater than 6, in the presence of clinical infection in an asthmatic, this is evidence of some condition tending to produce a long band, such as pulmonary fibrosis. An asthmatic with a comparable amount of infection and a Weltmann coaguation band of less than 6, probably has little pulmonary fibrosis. The Weltmann reaction, when correlated with other clinical and laboratory tests, gives information which is helpful in evaluating the presence of either infectious or noninfectious complicating conditions.

SUMMARY

1. The Weltmann reaction has been studied in a series of 224 asthmatics (51 children and 173 adults).

2. Patients were classified according to the clinical diagnosis into five groups: (1) asthma, (2) asthma and other allergy, (3) asthma with noninfectious diseases, (4) asthma with chronic, and (5) asthma with acute infections.

3. Patients with uncomplicated asthma, or asthma and another allergic disease had coagulation bands of 6, except six patients with intrinsic asthma due to bacterial allergy and intrapulmonary infections, who had bands of 5.

4. The asthmatics with noninfectious complications had bands of 7 or longer.

5. All patients with infection had bands less than 6, except for a group of twenty-four asthmatics with chronic infection. One-half of these patients had very mild infection. The other half had, in addition to infection, some marked degree of emphysema or pulmonary fibrosis.

6. On the basis of the Weltmann reaction, one may apparently detect the presence of infection and fibrotic change in asthmatics.

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DERMATITIS DUE TO RESIN-FINISHED SHORTS AND FABRICS

An Investigation of the Cause, Pathogenesis, and Related Phenomena Observed in Ten Cases

Harry Keil, M.D.
New York, N. Y.

In 1940 Schwartz and his co-workers[1] made an important contribution to the subject of dermatitis due to shorts and other fabrics. By means of patch tests, the cause was definitely established as present in the "finishing" material. Reasoning on the basis of certain analogies, these observers suggested that the etiologic agent was an "acid ester gum," in particular the free abietic acid found in this product. Subsequently the ester gum was stated to be glycerin abietate.[2] Shortly after, a few observers[3, 4] denied that abietic acid was the fundamental cause of this type of contact dermatitis, and preliminary evidence was given to indicate that glycerin abietate was probably not the ester gum responsible for dermatitis due to shorts and other fabrics.[4] In a recent publication[5] additional evidence was furnished which eliminated glycerin abietate as the cause and implicated another type of ester gum.

This report is based on a detailed study of ten cases of dermatitis due to shorts and similar fabrics; one of these (Case 6) was deemed worthy of a separate report.[5] This group comprised eight patients seen during the past nine months and two patients who had been afflicted with this type of contact dermatitis in 1940 and early in 1941, respectively. In order to show the progress of study, these protocols will be given in chronologic order of investigation.

In a previous publication[4] pertinent data on the chemistry of the various types of rosins and their derivatives were described. A recapitulation of some of the principles is essential to an understanding of the general problem. Rosin (gum or wood) is composed chiefly of abietic acid ($C_{19}H_{29}COOH$). When, under conditions of manufacture, it is reacted with glycerin (an alcohol with three available hydroxyl groups), glycerin abietate is formed. In the process an attempt is made to obtain the triglyceride of abietic acid, although small amounts of diglyceride and monoglyceride are formed, depending on the thoroughness of the esterification. To the product formed by the interaction of rosin and glycerin, the term ester gum, an inaccurate and confusing name, has been applied by the rosin industry. Since it is difficult, if not impossible, to esterify all the abietic acid, some of it is left uncombined.

From the Skin and Cancer Unit, New York Postgraduate Medical School and Hospital, Columbia University.

The amount of free acid, which is generally small, is designated by the "acid number." If, for example, the acid number is 6, this means that 6 mg. of potassium hydroxide will neutralize the free acid in 1 Gm. of resinous substance.

What has confused the issue is that there are now available numerous other derivatives of rosin which may be designated technically as ester gums. These new substances involve the interaction of rosin with monoatomic alcohols (methyl and ethyl alcohol), diatomic alcohols (diethylene glycol), tetratomic alcohols (pentaerythritol), and probably many other compounds with available hydroxyl groups. The abietates formed from these different alcohols are sold under commercial names, whereas the term ester gum is generally applied in the industry to glycerin abietate only. In this paper, the substance causing the dermatitis due to the resin finish in shorts and other apparel will be referred to as the special ester gum. All ester gums have an acid number which is determined in the same way as that for glycerin abietate. In addition, such compounds are often hydrogenated in order to eliminate, so far as is possible, the tendency to oxidation which is manifested by the double bonds in the structural formulas.

My observations indicate that the rosins and their derivatives are not primary irritants. This refers only to those which I have had the opportunity to study and which have been manufactured in recent years. However, substances of this class do have varying abilities to sensitize the skin. Under present conditions of industrial use, abietic acid seems to be an uncommon sensitizer; glycerin abietate, an occasional sensitizer; and the special ester gum causing the dermatitis produced by shorts and other fabrics, a more frequent sensitizer. It must be remembered, in evaluating the sensitizing power of the latter substance, that it is not used alone but rather in the presence of wetting agents which probably enhance contact with the integument.

CASE REPORT

CASE 1.—A man, 34 years old, bought three new pairs of shorts. He wore unwashed pair 1 continuously for two days, even during sleep. Then he switched to an old pair, and not until a week later did he wear unwashed pair 2. At the end of forty-eight hours, he had pruritus in the genital area. That night he wore washed pair 1 but, owing to intense itching, he was unable to sleep. Despite this, pair 1 was kept on continuously for the next two days.

Examination showed pronounced edema of the scrotum and penis, with erythema of the neighboring parts of the thighs. Pruritus was intense.

Patch tests, read in forty-eight hours, showed the following: unwashed shorts (pair 3), a pronounced vesicular and edematous reaction (3 plus); washed shorts (pair 1), a similar but less intense response (2 plus); crude abietic acid, mild erythema and edema (1 to 2 plus);

dammar resin, 1 plus. Glycerin abietate, two types of wood rosin, and gum rosin gave negative reactions. The sites of two different adhesive tapes showed only traumatic irritation. A third type of tape known to contain glycerin abietate did not affect the skin at all. Two days later, the reactions to the unwashed and washed shorts were still present in their former intensity, whereas the other mild positive reactions were fading. The irritations caused by the two adhesives were also disappearing.

Patch tests were made with a second series of substances: 50 per cent stock (probably gum) turpentine, partially purified abietic acid, crude rosin, and rosin residue. In forty-eight hours all produced varying degrees of simple erythema. The following day the site of the turpentine revealed a stronger reaction, consisting of erythema with rare vesicles (1 to 2 plus), the partially purified abietic acid showed a slightly more pronounced reaction (1 to 2 plus), and crude rosin and rosin residue each showed an indefinite plus-minus reaction.

A third series, consisting of sixteen different dyes, gave negative results. In places where the adhesive tape was creased, there was evidence of traumatic irritation, whereas the smoothly covered areas showed no such irritative redness. This illustrates the mechanical factor in traumatic irritation.

A fourth series consisted of glycerin, xylene and mineral oil in equal volumes, pure xylene (the patient was told to remove this on the slightest sign of irritation), and the following substances, dissolved in a small amount of xylene and each diluted with an equal volume of mineral oil: partially purified abietic acid, crude rosin, rosin residue, crude abietic acid (similar to that used by Neilson and Reiches[3]), and gum rosin. All gave negative reactions in forty-eight hours and remained so on succeeding days. It is interesting that these substances, though involving rosins, showed differences in solubility in xylene and that two of them, crude rosin and rosin residue, revealed the probable presence of oxidized matter which was insoluble in mineral oil. This study, although admittedly crude, indicated that the presence of xylene did not enhance the reactions of the skin to abietic acid and other resinous substances and that the negative responses were due to simple dilution.

Patch tests with pure amyl butyrate and small amounts of glycerin abietate, crude abietic acid, and partially purified abietic acid dissolved in this solvent (clear solutions) were all negative. Similar results were obtained in a series consisting of 1 per cent ammonium hydroxide and the aforementioned resins added to this solution; glycerin abietate (not very soluble); crude abietic acid (more readily soluble with production of a foamy, soapy solution containing some precipitate); and partially purified abietic acid (a thick, soapy solution with suspended coarse particles).

Discussion.—The interval between the first wearing of the unwashed shorts and the onset of symptoms could be accurately determined as

eleven days. At the end of this time the patient was unable to wear the shorts, even though washed.

None of the various samples of rosin or its derivatives gave as intense a reaction in patch tests as that shown by the unwashed shorts, despite the application of these substances *undiluted*. Despite the mild sensitivity to crude abietic acid and dammar resin in the concentrations used, these substances could not possibly be the cause of the dermatitis due to the shorts. The negative response to undiluted glycerin abietate eliminated this substance as the cause, and further substantiation was provided by the negative response to a specimen of adhesive tape known to contain this chemical as its sole resinous ingredient. In addition, two other adhesive tapes showed only the traumatic irritation observed in many persons.

Definite hypersensitivity was manifested to 50 per cent stock (probably gum) turpentine and to undiluted partially purified abietic acid, but these were not causally related to the clinical dermatitis.

The presence of xylene did not enhance the reactions to the various specimens of abietic acid. On the contrary, the reactions became negative, due to the effect of simple dilution. Dissolving glycerin abietate and partially purified abietic acid in amyl butyrate and in 1 per cent ammonium hydroxide resulted in no increase in the action of these resins on the skin.

Consequently, the studies indicated that the precise etiologic agent had not yet been found.

Case 2.—This was a typical example of dermatitis due to shorts. Patch tests gave erythemato-vesicular reactions to the fabrics. A limited investigation of this case revealed three points of interest: (1) undiluted partially purified abietic acid gave an entirely negative patch test, thus eliminating this substance as the cause; (2) the skin was unaffected by one adhesive tape and showed ordinary traumatic irritation to a second; (3) two days after the patch tests with the fabrics were applied, there was a focal reaction on the thighs and buttocks.

Case 3.—This patient, a young man, had purchased four pairs of shorts of one grade and four pairs of another. Three pairs of each type were worn unwashed and on the seventh day he had itching of the scrotum. The patient perspired much. Up to this time he had not worn the shorts during sleep but, puzzled by the symptom, he decided to try this; whereupon the eruption spread to the thighs down to the popliteal spaces.

Examination revealed an eczematous eruption on the scrotum. There were bright red papules in the area covered by the shorts, with similar lesions along the posterior aspects of the thighs down to the popliteal spaces, and an eczematous patch over the lower sacral region.

Patch tests showed variable reactions to the shorts. The most pronounced reaction was given by one pair of washed shorts (vesicular

response with huge edema, graded 4 plus), whereas three other pairs of the same make showed negative results in the washed and unwashed state. The second type of shorts gave a less intense positive reaction to two pairs which had been washed and a negative response to the remaining pairs which were washed and unwashed, respectively. Under the same manufacturer's name both good and bad fabrics were being sold at the same time.

A second series of patch tests, also read in forty-eight hours, revealed the following: undiluted partially purified abietic acid and crude abietic acid, each a questionable reaction consisting of erythema with rare vesicles; undiluted wood rosin (purified), a 3 plus reaction; wood rosin FF grade, a slightly more intense reaction graded 3 plus also; a type of hydrogenated rosin, a 2 plus reaction; gum rosin, 2 to 3 plus reaction; glycerin abietate, a pronounced vesicular response, 4 plus; a type of adhesive tape known to contain glycerin abietate, a 3 plus reaction; another type of adhesive tape, ordinary traumatic irritation; and still another variety of tape known to contain a dehydrogenated rosin, a vesicular reaction, 3 plus. Two days later the sites of the crude abietic acid, the partially purified abietic acid, and the gum rosin were fading, whereas the remaining reactions, including those shown by the two adhesive tapes to which the patient was hypersensitive, were still present in their former intensity. The site of traumatic irritation to the third adhesive tape was no longer visible.

A third series of patch tests, also read in forty-eight hours, revealed the following: 50 per cent stock (probably gum) turpentine, negative; the dehydrogenated rosin, known to be present in one of the adhesive tapes to which he was sensitive, an intense vesicular reaction (graded 4 plus); the same dehydrogenated rosen dissolved in xylene and diluted with an equal volume of mineral oil, only a 1 to 2 plus reaction (dilution effect); glycerin abietate dissolved in the same way, a 2 plus reaction (again a dilution effect); crude abietic acid and partially purified abietic acid dissolved in the same way, negative reactions. It may be noted that the partially purified abietic acid was now colored yellow, indicating the presence of oxidized products.

A fourth series of patch tests, read in twenty-four hours, revealed the following: pure methyl abietate, a vesicular and edematous reaction (3 plus); abietic acid ester of pentaerythritol, a 2 plus reaction; a type of hydrogenated rosin, negative; another grade of wood rosin FF, negative; and a wood rosin containing phenolic bodies (1 plus). On the next day the site of methyl abietate showed small bullae (4 to 5 plus); the other ester gum was slightly more vesicular than before (2 to 3 plus); the wood rosin containing phenolic bodies remained the same; and both the wood rosin FF and the hydrogenated rosin were still negative.

A fifth series involved the dilution of methyl abietate with acetone, using concentrations of 50, 25, 12, 6, and 3 per cent by volume of this

substance in acetone. The cutaneous responses diminished in intensity with the decrease in concentration, but the patient's skin was so hypersensitive that even 3 per cent by volume of methyl abietate in acetone gave a 2 plus reaction.

Discussion.—The skin of the patient was hypersensitive to a number of ester gums, such as glycerin abietate, methyl abietate, and the abietic acid ester of pentaerythritol. Of these, the reactions to methyl abietate were the most pronounced; yet, this was probably not the cause of the dermatitis due to shorts, as will be indicated by other data to be recorded. All the positive reactions to these ester gums were group responses.

In addition to these group reactions, hypersensitivity was manifested to other resinous substances; for example, a type of dehydrogenated rosin, gum rosin, and a variety of wood rosin FF. Contrariwise, there were negative responses to a type of hydrogenated rosin and a wood rosin FF obtained from another manufacturer. These results indicate the care required in deducing the precise etiology in the presence of group reactions and of complex resinous products apparently of the same type.

Since a specimen of wood rosin FF of one grade gave a positive reaction, whereas another sample, similarly graded, showed a negative response, it seemed unlikely that abietic acid, the major component of both, was the irritant substance. It is possible that the former contained some other ingredient responsible for the positive reaction. The undiluted crude abietic acid and the partially purified abietic acid gave only questionable reactions which could in no way be compared with those elicited by the shorts; moreover, dissolving them in xylene and mineral oil, far from enhancing their action on the skin, resulted in entirely negative responses, showing the effects of simple dilution.

Studies made with methyl abietate, diluted with acetone, showed that the presence of this solvent did not enhance the penetrability of this ester gum and that the reactions elicited were proportionate to the concentration of the methyl abietate. Similar observations were made in the case of glycerin abietate, dehydrogenated rosin, and other rosins dissolved in a mixture of xylene and mineral oil.

Of the three types of adhesive tape used, the patient was sensitive to two. This hypersensitivity was shown to depend chiefly on their resinous ingredients; in the one case, glycerin abietate; in the other, a form of dehydrogenated rosin. The negative response to the third type of adhesive indicated that it contained some other type of resinous substance to which the patient was not sensitive, or that its concentration in the formula was too small to elicit a response. This conclusion could be correlated with the points that the patient was not sensitive to all the resinous compounds used in this study, and that some of the mild reactions with substances in the undiluted state could be eliminated by dilution.

It is interesting that in this case there was no hypersensitivity to 50 per cent stock (probably gum) turpentine.

The spread of the eruption after wearing the shorts during sleep was probably caused by local absorption of the offending substance due to greater opportunity for contact.[5] Had the patient continued this practice, there is little doubt that the eruption would have spread to larger tracts of the body.

CASE 4.—A middle-aged man wore three pairs of unwashed shorts, following which the penis and the adjacent parts became red, swollen, and itchy. The attack lasted one week.

Patch tests, read in forty-eight hours, showed the following: unwashed shorts, an intense vesicular reaction (3 plus); washed shorts of the same grade, negative; partially purified abietic acid, a questionable reaction consisting of mild redness and rare papules; and negative responses to crude rosin, rosin residue, gum rosin, crude abietic acid, and glycerin abietate. Crude abietic acid, partially purified abietic acid, and glycerin abietate, each dissolved in xylene and diluted with an equal volume of mineral oil, gave negative reactions. The control solution of xylene and mineral oil in equal parts was also negative. Three types of adhesive tape showed only mild traumatic irritation.

Subsequently, he wore a thoroughly washed pair of shorts by error and experienced no ill effects therefrom. This was the fabric to which he had given a negative patch test. I am not certain of whether this material had been finished in the same way as the shorts to which he had reacted strongly or of whether, by thorough washing, all the resinous finish had been removed.

Discussion.—The clinical features in this case indicated a mild hypersensitivity to shorts. The patient was not sensitive to pure glycerin abietate and only showed mild traumatic irritation to an adhesive tape which contained this substance. The questionable positive reaction to undiluted partially purified abietic acid could be eliminated entirely by simple dilution. The presence of xylene did not enhance the effects of glycerin abietate, crude abietic acid, and partially purified abietic acid on the skin.

CASE 5.—A man, 19 years old, wore a new pair of unwashed shorts. Three days later he noted a rash about the genitals. Examination showed a dry erythemato-papular and scaling eruption on the penis, genito-crural areas, thighs and buttocks. In one week the lesions were practically gone.

Patch tests, read in forty-eight hours, showed the following: two types of washed shorts, each a 1 plus reaction; methyl abietate 50 per cent by volume in xylene, an edematous reaction spreading beyond the original site of the patch (graded 2 to 3 plus); abietic acid ester of pentaerythritol, a 1 to 2 plus reaction; glycerin abietate, a 1 plus reaction; gum rosin, a form of hydrogenated rosin, and levo-pimaric acid,

each a plus-minus reaction; and completely negative responses to dehydroabietic acid, lactone of 13-hydroxytetrahydroabietic acid, another form of hydrogenated rosin, partially purified abietic acid, crude rosin, wood rosin FF, wood rosin containing phenolic bodies and dammar resin. Two types of adhesive tape showed only mild traumatic irritation.

Three days later the sites of the shorts showed pigmentation. All the other reactions had disappeared, except for remains at the sites of methyl abietate and abietic acid ester of pentaerythritol. One of the adhesive tapes showed occasional pigmented stripes, this being the tape which contained the hydrogenated rosin giving the plus-minus reaction.

Discussion.—The washed shorts gave mild, though definite, positive reactions. Possibly more thorough washing might have produced negative responses, and this possibility must be borne in mind in making patch tests. There were group reactions to methyl abietate, abietic acid ester of pentaerythritol and glycerin abietate, but these were not especially pronounced. The mildness in the cutaneous reactions to all the rosins was shown by the diminished intensity three days later. The occurrence of the group reactions argued strongly for the diagnosis of dermatitis due to shorts. If the incubation period of three days is correct, there is a strong possibility that the patient might already have come in contact with the "special ester gum" in some other pair of shorts. It is hard to believe that the hypersensitivity to the shorts, which was not especially intense, was based on a previously acquired sensitivity to adhesive tape alone, although this is remotely possible. It may be pointed out that the skin gave negative reactions to partially purified abietic acid and many other rosins in which this acid is the principal ingredient.

Case 6.—In this case of dermatitis due to resin-finished facing on cotton underwear, patch tests could be made for the first time with the special ester gum responsible for this general type of contact dermatitis. Only the salient features will be mentioned here, as the details have been recorded in a separate publication.[5]

A man, 63 years old, had two attacks of swelling of the penis and scrotum. These attacks were separated by an interval of one and one-half years; the last one occurred twelve hours after contact with the fabric.

Patch tests, read in forty-eight hours, showed pronounced vesicular reactions (3 to 4 plus) to the resin-finished facing of two pairs of unwashed cotton underwear. There were group reactions to methyl abietate 50 per cent by volume in xylene (3 plus), pure glycerin abietate (2 plus), and abietic acid ester of pentaerythritol (2 plus). Lesser degrees of positive responses were shown to partially purified abietic acid, two types of wood rosin, and two varieties of hydrogenated rosin.

The skin was hypersensitive to two types of adhesive tape and negative to a third.

Patch tests with various dilutions of methyl abietate in xylene showed that the intensity of reaction was dependent on the concentration of this ester gum.

Patch tests with the special ester gum dissolved in xylene indicated a remarkable hypersensitivity to this substance. Thus, a contact of only five minutes showed, forty-eight hours later, intense vesicular and edematous reactions, accompanied by a "toxic" erythema in the neighboring areas of the arm to which these patches had been applied, and also in the corresponding forearm. This was caused by local absorption of the special ester gum. Patch tests with various dilutions of this substance, dissolved in mixtures of xylene and mineral oil, gave unusually intense reactions; even an approximate concentration of 0.8 per cent by weight in xylene and mineral oil revealed a 3 plus vesicular response, whereas a 16 per cent concentration in these diluents resulted in a reaction graded as 4 to 5 plus.

CASE 7.—A man, 34 years old, had an itchy eruption on the thighs and perineum some two years before the present observation. At that time he had worn, at irregular intervals, several new pairs of shorts, both washed and unwashed. A subsequent examination showed an erythema about the thighs and buttocks. The patient did not return for patch testing with his shorts.

On Feb. 5, 1942, I saw him for the first time. There was a papulo-urticarial eruption on the outer aspects of the thighs. Of the routine patch tests with fifteen substances, only paraphenylendiamine gave a mild positive reaction. A patch test with unwashed shorts (purchased two years before) showed, in forty-eight hours, a vesicular and edematous reaction (3 plus). Two different adhesive tapes were well tolerated, except that one showed transient traumatic irritation.

Additional patch tests, read in forty-eight hours, showed the following: another pair of unwashed shorts (also purchased two years before), a vesicular and edematous reaction (3 plus); a similar type of shorts that had been repeatedly washed, negative; new shorts recently purchased, negative; shorts that had been worn during the past three years without ill effects, negative; a third type of adhesive tape containing glycerin abietate, mild traumatic irritation; a hydrogenated rosin, plus-minus; and negative responses to undiluted samples of levo-pimaric acid, dehydroabietic acid, lactone of 13-hydroxytetrahydroabietic acid, partially purified abietic acid, glycerin abietate, gum rosin, wood rosin FF, wood rosin containing phenolic bodies, another type of hydrogenated rosin, methyl abietate 50 per cent in xylene, abietic acid ester of pentaerythritol, and dammar resin.

The same day fresh patches of the special ester gum were applied in the following concentrations in xylene: 32, 16, 6.4, 3.2, and 1.6 per cent

by weight. After five minutes these had to be taken off due to sensations of burning. There was mild redness at the sites, probably an effect of the xylene itself. The same solutions were applied to the opposite arm, but this time each was diluted with an equal volume of mineral oil, thus reducing the concentrations by approximately one-half. As a control, an equal volume of xylene in mineral oil was used. The next day the areas where the ester gum in xylene alone had been applied for only five minutes were entirely negative. On the opposite arm the first three highest concentrations (approximately 16, 8, and 3.2 per cent by weight in xylene and mineral oil) showed papular erythemas, with diminishing intensity respectively, whereas the two lowest concentrations (1.6 and 0.8 per cent by weight) and the control (xylene and mineral oil in equal volumes) were negative.

Because the reactions were mild, the special ester gum was applied in a concentration of 40 per cent by weight in xylene and mineral oil. The next day this patch showed a 2 plus reaction, consisting of edema and some vesicles. Two days later the reaction was more pronounced, and at this time (four days after the original dilutions of this ester gum had been applied), the first three highest concentrations showed more intense reactions also. At the end of ten days all the sites were no longer visible.

Discussion.—This was an example of dermatitis due to shorts purchased about two years before the present observation. Had patch testing been limited to the one pair of thoroughly washed shorts, the status of the case would have been doubtful. The clinical features indicated a relatively mild degree of hypersensitivity, and the results of the patch tests substantiated this view.

Group reactions to other ester gums were absent and the patient was able to tolerate three different types of adhesive tape. Patch tests with the special ester gum to which he was sensitive showed that the intensity of the reactions depended on the concentration of this substance in xylene and mineral oil. When low concentrations (approximately 0.8 and 1.6 per cent by weight in xylene and mineral oil) were used, the tests were negative; with higher concentrations (3.2, 8, 16, and 40 per cent by weight in the same diluents), the intensity of the reactions, which increased in succeeding days, paralleled the concentration of the ester gum. In comparison with the patient in Case 6, an example of extreme hypersensitivity, the reactions were relatively weak and, in further contrast, the patient could tolerate for five minutes, without ill effects, the patch tests with the ester gum in xylene alone.

The response to the special ester gum, even in a concentration of about 40 per cent by weight in xylene and mineral oil, was less pronounced than that shown by the unwashed shorts. It is probable that this difference in reaction was due to the increased penetrability of the special ester gum owing to the nature of its physical state in the shorts.

CASE 8.—A man, 39 years old, complained of severe swelling of the penis, scrotum, and adjacent thighs. The affection had been present many weeks, but precise data were not available.

Patch tests, read in forty-eight hours, showed the following: washed pajamas, a vesicular and edematous reaction (3 plus); 50 per cent methyl abietate by volume in xylene, and a type of hydrogenated rosin, each 2 plus; abietic acid ester of pentaerythritol, glycerin abietate, partially purified abietic acid, crude rosin, and another type of hydrogenated rosin, each 1 to 2 plus; wood rosin FF and wood rosin rich in phenolic bodies, each 1 plus; and negative responses to dammar resin, gum rosin, levo-pimaric acid, dehydroabietic acid, and the lactone of 13-hydroxytetrahydroabietic acid. Of two adhesive tapes, only the one containing the hydrogenated rosin which gave a 1 to 2 plus reaction showed evidence of mild hypersensitivity.

Subsequently, patch tests with the special ester gum were applied in the following approximate concentrations: 0.8, 1.6, and 3.2 per cent by weight in xylene and mineral oil. In addition, 33 per cent by volume of hydrogenated methyl abietate in mineral oil was also applied. At the end of twelve hours, only the highest concentration of the special ester gum showed a faint erythemato-papular reaction (plus-minus), whereas the hydrogenated methyl abietate gave an erythemato-vesicular reaction extending beyond the zone of the patch site (2 to 3 plus). Two days later there were delayed reactions as follows: 0.8 per cent special ester gum, a pronounced papular reaction (1 to 2 plus); 1.6 per cent, a more intense response with a vesicular component (2 to 3 plus); and 3.2 per cent, a pronounced vesicular reaction (3 plus). The site of the hydrogenated methyl abietate was now fading. Two additional adhesive tapes showed only traumatic irritation. One week later all the reactions to the special ester gum had disappeared.

Discussion.—The delayed reactions to the low concentrations of the special ester gum were noteworthy. There was evidence of group responses of moderate degree to other ester gums. The patient seemed hypersensitive to only one of the four different adhesive tapes used, and this contained the hydrogenated rosin to which a 1 to 2 plus reaction had been given in the undiluted state.

CASE 9.—A man, 61 years old, had a diffuse scaling eruption on the genital areas, thighs, and flexor aspects of the forearms. He had worn a new pair of unwashed shorts, and the dermatosis had been present for some three weeks. A patch test with washed shorts gave a 1 to 2 plus reaction.

About one and one-half years later I was able to study the case in greater detail. Patch tests, read in twenty-four hours, showed negative responses to levo-pimaric acid, dehydroabietic acid, the lactone of 13-hydroxytetrahydroabietic acid, partially purified abietic acid (colored yellow), wood rosin FF, wood rosin rich in phenolic bodies, gum rosin,

two types of hydrogenated rosin, glycerin abietate, abietic acid ester of pentaerythritol, and methyl abietate 50 per cent in xylene. Two adhesive tapes also gave negative reactions.

On the same day fresh patches of approximately 0.8, 1.6, and 3.2 per cent by weight of the special ester gum in xylene and mineral oil were applied, together with 33 per cent by volume of hydrogenated methyl abietate in mineral oil. The next day these patch tests were negative, but there was a delayed papular reaction to the methyl abietate in the first series. A fresh patch of 16 per cent by weight of the special ester gum in xylene and mineral oil was placed on the skin of the opposite limb. Twenty-four hours later there was a delayed, mild papular reaction to the special ester gum in 3.2 per cent concentration, whereas the site of the 16 per cent concentration showed an immediate response, consisting of moderate edema and a few papules. Three days later, the latter revealed a more intense papular reaction (2 plus) and the 3.2 per cent concentration, a definite 1 plus reaction. There was evidence of mild hypersensitivity to a third type of adhesive tape.

Discussion.—This case illustrated a mild grade of group reaction to methyl abietate and the special ester gum. The former gave a delayed reaction, whereas the latter showed both delayed and immediate responses, depending on the concentration. At a concentration below 3.2 per cent by weight (approximate) in xylene and mineral oil, there were no reactions; 3.2 per cent, a delayed response; and 16 per cent, an immediate response. No group reaction to glycerin abietate occurred. Only one of the three adhesive tapes showed a reaction indicating a mild degree of hypersensitivity, which was difficult to differentiate from ordinary severe traumatic irritation. Of interest was the positive patch test to methyl abietate and the negative response to hydrogenated methyl abietate.

CASE 10.—A man, 50 years old, had edema and redness of the penis and scrotum, with scattered lesions in the "shorts area." Itching was severe. He had worn new unwashed shorts. Patch tests showed a 2 to 3 plus reaction to one type of shorts and only a 1 plus reaction to another type. No other details were available.

One year later I was able to study the case in greater detail. Patch tests, read in forty-eight hours, showed the following: glycerin abietate, erythema with faint vesicles (2 plus); methyl abietate 50 per cent by volume in xylene, many vesicles (3 plus); a type of hydrogenated rosin, faint redness (plus-minus); abietic acid ester of pentaerythritol, redness with faint vesicles (about 2 plus). Negative responses were obtained with dehydroabietic acid, levo-pimaric acid, lactone of 13-hydroxytetrahydroabietic acid, wood rosin FF, wood rosin rich in phenolic bodies, partially purified abietic acid (colored yellow), gum rosin, and another type of hydrogenated rosin. Two different adhesive tapes showed only traumatic irritation.

The same day additional patches of 0.8, 1.6, and 3.2 per cent by weight approximately of the special ester gum in xylene and mineral oil were placed on the skin of the right arm, together with 33 per cent by volume of hydrogenated methyl abietate in mineral oil. Twenty-four hours later, there were only questionable erythemas at the sites of the two highest concentrations of the special ester gum and the hydrogenated methyl abietate. At this time the previous positive reactions to the other ester gums (placed on the back) had diminished in intensity, and the adhesive irritations were no longer visible. A fresh patch of 16 per cent by weight approximately of the special ester gum in xylene and mineral oil was placed on the skin of the opposite arm.

The following day there were delayed reactions at the sites of the special ester gum: 0.8 per cent, a bright erythema; 1.6 per cent, an erythematous, edematous, and slightly vesicular reaction; 3.2 per cent, a widely distributed vesicular response. The site of the hydrogenated methyl abietate showed only slight redness. At this time, the area of the 16 per cent special ester gum was negative. Two days later the results were decisive: 0.8 per cent special ester gum, a mild erythema; 1.6 per cent, pronounced redness, edema, and vesiculation (2 to 3 plus); 3.2 per cent, a more widely distributed vesicular response (3 plus); 16 per cent, much itching and more intense vesiculation (3 to 4 plus). The site of the hydrogenated methyl abietate was negative. The weather at this time had been warm, and this may have aided in bringing out the reactions.

Discussion.—This patient illustrated a moderate degree of group reactions to various ester gums. Patch tests with many types of rosins and partially purified abietic acid (colored yellow) were negative. Notable were the delayed positive responses to the small concentrations of the special ester gum, the intensity of the reactions being dependent on the concentration of this substance.

GENERAL DISCUSSION

The protocols recorded in this paper illustrate the principles which have been discussed in another publication.[5] Here, only a few additional questions of fundamental interest will be considered.

The evidence presented indicates that neither abietic acid nor glycerin abietate was the cause of the contact dermatitis due to certain resin-finished fabrics. The etiologic agent was a special ester gum. The results of patch tests with this substance depended on two general factors: (1) the patient's degree of hypersensitivity; and (2) the concentration of the substance. Thus, in a person with pronounced hypersensitivity (Case 6), small concentrations of the special ester gum were sufficient to produce immediate intense vesicular responses. Contrariwise, in those with low degrees of hypersensitivity (Case 7), negative reactions were elicited with small concentrations of the special

ester gum, delayed responses with higher concentrations, and immediate reactions with still higher concentrations. Between these extremes were those showing intermediate grades of response in patch tests (Cases 8, 9, and 10). Moreover, the type of reaction seemed to be dependent on whether it was immediate or delayed; in the former case, generally a vesicular reaction, occasionally an intense papular or papulovesicular response; in the latter case, an erythematous or papular reaction. Finally, it was a general rule, with some exceptions, that an intense response to the patch test with the special ester gum was accompanied by group reactions to other types of ester gums.

There remains to be considered the important question of the physical state of the special ester gum in the finish. The available data indicate that this substance exists in a water-miscible, emulsified form, produced by the introduction of a wetting agent, such as lauryl sodium sulfate or triethanolamine oleate. In this emulsified state this substance apparently penetrates the interstices of cloth more readily, a physical attribute that explains or sheds light on many clinical phenomena. Since the emulsion is water-miscible, this explains why cases are more commonly seen in a severe form in the summer when patients perspire profusely. The differences in intensity of reactions shown by patch tests with the special ester gum dissolved in a mixture of xylene and mineral oil and those revealed by pieces of the fabric itself may be due to the influence of the wetting agent on the physical state of the substance as it comes in contact with skin. It is not yet clear whether the presence of a wetting agent permits direct penetration into the epidermis itself, since wetting and penetration are not necessarily identical properties. The frequent follicular morphology of lesions may be attributed, in all likelihood, to penetration of the emulsion into the follicular orifices. Owing to my inability to obtain the original emulsion, I was unable to determine whether the presence of the wetting agent enhanced the cutaneous effects of the special ester gum simply by the change in physical state of the latter substance or by an actual increase in the penetrating power of the mixture.

Repeated washing of the fabric will dissolve out a great deal of the emulsion containing the resinous substance; and in some cases, especially those manifesting low degrees of hypersensitivity, patch tests with the suspected underwear may be negative or almost negative. A negative response to a thoroughly washed fabric does not therefore eliminate this material as the cause, but such a result is decisive when *unwashed fabrics* are used in patch testing. The population is still exposed to shorts containing such resinous emulsions, and these fabrics are still being sold along with others devoid of such finishing material. Whether the former represents old stocks or whether the practice is still being continued on a limited scale, owing to ignorance of the potential senitizing power of such emulsions, I do not know.

SUMMARY

From these data the following general principles may be derived:

1. Fabrics covering large tracts, or relatively large tracts, of the body should not be finished with mixtures containing resins, particularly in the presence of a wetting (emulsifying) agent, unless it can be shown that such mixtures have negligible sensitizing powers.

2. This applies not only to the special ester gum, but also to other substances with similar physical properties.

3. General methods of patch testing with processed wearing apparel have been previously discussed.[6]

My thanks are due to Dr. Louis Frederick Fieser, through whose kindness I obtained some of the substances used in this study.

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Special Reports

THE USE OF AMINOPHYLLINE RECTAL SUPPOSITORIES IN THE TREATMENT OF BRONCHIAL ASTHMA

Preliminary Report

Susan C. Dees, M.D.
Durham, N. C.

THE literature contains numerous reports on the intravenous and oral use of aminophylline (theophylline ethylenediamine) in the treatment of bronchial asthma.[1-7] The rectal administration of aminophylline for relief of bronchial asthma has not been stressed. The drug has been found to be effective when given by rectum as an instillation, or suppository, in the treatment of Cheyne-Stokes respiration and cardiac asthma, or for use as a diuretic.[8-10] After observing the favorable effects of aminophylline in bronchial asthma, when given either by the oral or intravenous route, we investigated the clinical effectiveness of a rectal suppository containing 0.25 Gm. of aminophylline in cocoa butter with 5 or 7 per cent wax, and have found this to be a very satisfactory therapeutic aid in securing prompt and prolonged relief of asthmatic symptoms.

Clinical trial has been made in fifty-five severe asthmatics: forty-nine adults, and six children. These patients were selected because they had severe and, at times, intractable asthma. The clinical diagnosis was asthma of mixed type, combining both intrinsic and extrinsic factors in the majority of patients. Desensitization to specific allergens or to autogenous vaccines had been attempted, or was being carried out, in 60 per cent of the group. In addition to desensitization, it was necessary in all of the group to have frequent or almost continuous recourse to symptomatic medication. Fifteen individuals used epinephrine hydrochloride 1:1,000 hypodermically, or adrenalin in oil intramuscularly, at least several times every day. Eleven had become adrenalin-fast. Of the entire group, many had acquired a tolerance to oral aminophylline or ephedrine, or had developed sensitivity to these drugs, so that their original effectiveness in relieving symptoms was either lost or overshadowed by the unpleasant side effects which accompanied their use.

Forty of these patients were treated successfully with intravenous aminophylline (0.24 to 0.48 Gm. in 10 to 20 c.c. of physiologic saline or glucose solutions), at one or more times during the period they were

From the Departments of Pediatrics and Medicine, Duke University School of Medicine and Duke Hospital, Durham, N. C.

followed. In spite of the fact that intravenous administration was very slow, we have encountered several instances of severe cardiovascular reactions among these patients. It is our experience that any degree of right-sided cardiac failure is a definite contraindication to the intravenous use of the drug. In addition to the possibility of severe reactions, intravenous medication often is inconvenient for an asthmatic to obtain, since it must be given by a physician, and frequently is obtainable only for hospitalized patients.

Aminophylline rectal suppositories were first used in this clinic in hospitalized asthmatics with severe attacks, ten of whom were in status asthmaticus. It was found that symptoms disappeared on an average of twenty minutes after administration, and the relief lasted from four to twenty-four hours. Ambulatory patients reported similar results. The treatment was well tolerated by six asthmatic children. Fig. 1 shows the relative effectiveness of the suppositories as compared with other medications.

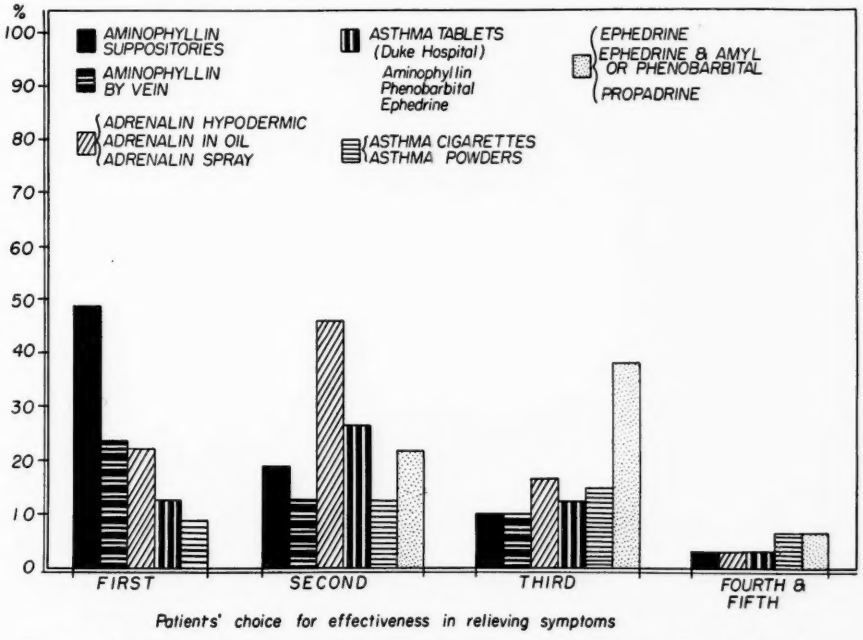

Fig. 1.—Relative effectiveness of suppositories as compared with other medication.

Eight patients reported no relief of symptoms by the suppository. One patient with severe hemorrhoids complained of rectal burning, and one patient six weeks pregnant believed the suppositories produced nausea. Two other adults stated that the suppositories acted as a "laxative" and were difficult to retain. No other ill effects were noted or reported. There was no evidence of any form of vascular reaction or any symptoms of gastric or intestinal irritation.

The suppositories, containing 0.25 Gm. of aminophylline, usually are prescribed for use at bedtime, and not oftener than once every six hours during the day. On this routine, it has been possible to maintain severe asthmatics free of symptoms during the entire day and night. Ordinarily, two suppositories, one in the morning and one in the evening, are adequate. We have found that rectal aminophylline frequently acts similarly to its intravenous use in restoring adrenalin sensitivity to the adrenalin-fast patients.

Aminophylline by other routes, and other antispasmodic drugs, can be used in combination with the suppositories, if desired. We have confined our preliminary trial of aminophylline suppositories to the severest cases of asthma in our clinic. In these patients it has been very effective. It fills the need for a drug which will act rapidly over a long period of time and can be easily self-administered.

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PLASMA TREATMENT OF SEVERE, NEAR-FATAL ANAPHYLACTIC SHOCK

ARTHUR H. RAYNOLDS, M.D.
NEW YORK, N. Y.

BLOTTNER[1] recently reported a case of severe exudative anaphylactic shock with hemoconcentration in a patient whom he treated intravenously with saline solution. He emphasized that the loss of fluid into subcutaneous and other tissues might lead to a condition not unlike that encountered in surgical shock and suggested that plasma might be more useful than saline solution.

We were fortunate recently in seeing a somewhat similar case, and in treating the patient with plasma intravenously. The result was so favorable it would appear that in plasma we may have a specific therapy for severe exudative anaphylactic shock associated with hemoconcentration and loss of blood volume.

CASE REPORT

A. V., a 40-year-old white woman of Italian extraction, has been a patient in the allergy clinic for nine years under treatment for hay fever and asthma. She was known to be sensitive to ragweed, oak, elm, and house dust, and had been receiving immunizing injections for ragweed, oak, and elm. At about 2:30 P.M. she received her injections, and within two to three minutes complained of choking, watering eyes, and itching underarms. She was immediately given 0.5 c.c. of epinephrine 1:1,000 subcutaneously, with moderate immediate relief. Shortly thereafter, her symptoms recurred, and epinephrine 1:1,000, 0.5 c.c., was given subcutaneously again. There was little improvement, and aminophyllin, 0.24 Gm., was given intravenously. At this time, about one and one-half hours after her immunizing injections, her arms and legs were noted to be diffusely swollen, with a great many wheals of varying sizes completely covering them. The face was edematous. The blood pressure was found to be 78/40 mm. Hg. An additional 0.25 c.c. of epinephrine was given subcutaneously. Ten minutes later the systolic blood pressure was faintly heard at 50 mm., the diastolic being unobtainable. Itching become intense. At this time, for a brief moment, the patient sat up and immediately fell backward in syncope. Epinephrine 1:1,000, 0.5 c.c., was again given subcutaneously, the total now being 1.75 c.c. There was no evidence at this time of asthma, rhinitis, or glottal edema. Her only complaints now were weakness, nausea, and itching. The blood pressure continued to drop, entirely unaffected by epinephrine or aminophyllin. Because her condition was becoming increasingly precarious, she was hospitalized for further treatment.

From The New York Hospital and Department of Medicine, Cornell University Medical College.

At about 5:30 P.M. she was given an infusion of 100 c.c. of normal saline, immediately followed by plasma, with prompt evidence of improvement, the blood pressure rising to 90/60 mm. Hg within thirty minutes. As the last portion of plasma was running in, the patient began to have chills, and further administration was stopped. About 500 c.c. of plasma were given altogether. The chills lasted two hours. The blood pressure remained at 96/60 mm. Hg, and it was evident that she was recovering. Moderate asthma, rhinitis, and urticaria appeared later but were of no consequence as regards the outcome, as they were now easily controlled by epinephrine. Examination of the blood showed only mild hemoconcentration, since it was done after the administration of plasma. She continued to improve rapidly and left the hospital approximately forty-eight hours after admission.

SUMMARY

1. A report is presented of a case of severe, near-fatal anaphylactic shock of "exudative" type (excessive blood volume loss).

2. The intravenous use of plasma, now increasingly available, may prove to be a specific treatment for this alarming condition. It should be tried early and in adequate amounts.

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Society Proceedings

SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

ANNUAL MEETING, NEW YORK, N. Y., DEC. 5, 1942

Blood Pressure Fluctuations in Bronchial Asthma. Howard Osgood, M.D., Buffalo, N. Y. (Abstract.)

In recent years there has been considerable discussion of the question as to whether or not the mean systolic blood pressure shows a sustained rise or fall during an attack of asthma. Of more interest and significance, however, are certain fluctuations of the systolic blood pressure which occur with each respiratory cycle during the asthmatic paroxysm.

When the paroxysm is severe, these fluctuations may be of sufficient magnitude to produce a noticeable waxing and waning of the radial pulse to palpation. A more common finding, however, is a lesser degree of fluctuation, not appreciable at the wrist, but readily detected by the use of a sphygmomanometer.

In healthy individuals, on quiet breathing, there is a normal fluctuation of the systolic pressure of 2 mm. to 6 mm. Hg, with the high point coming during expiration and the low point during inspiration.

From observations carried out over three years, I have found that, in asthmatic patients free for the time being of bronchial obstruction, the respiratory fluctuation is quite normal, both as to amplitude and as to timing of the high and low points during the respiratory cycle. An exception to this finding may be noted in certain chronic asthmatic patients exhibiting some persistent wheezes but with little apparent dyspnea; the respiratory fluctuation may be somewhat increased under these circumstances.

During an asthmatic paroxysm, the respiratory systolic fluctuation is consistently elevated and may range from 12 to as high as 45 mm. with each respiratory cycle. The peak, in my experience, uniformly comes at some point during expiration, and the trough during inspiration. This heightened fluctuation persists as long as the asthma continues, and parallels closely the varying severity of the dyspnea. When there seems to be a discrepancy between the physical findings of bronchial obstruction in the lungs, on the one hand, and the apparent respiratory distress, on the other, I have found that a determination of this respiratory fluctuation has yielded a quite accurate estimate of the actual degree of respiratory obstruction present.

When palliative treatment with epinephrine or aminophylline affords relief, the respiratory fluctuation coincidentally decreases to normal or near normal limits.

When palliative treatment does not afford relief, the respiratory fluctuation continues to be large.

From some experimental work with respiratory obstruction on cats and on healthy men, and from a study of the pertinent literature, I believe that the increased respiratory fluctuation of the systolic blood pressure in asthma is a reflection of some disturbance of the blood flow to the right side of the heart and through the pulmonary circuit, induced by the excessive changes of the intrathoracic pressure present during respiration in bronchial asthma.

The parallelism between the amplitude of this respiratory fluctuation and the severity of the asthma has been close enough so that it may be taken as an indirect and approximate measure of the degree of obstruction present. Conversely, observations on the behavior of this fluctuation under various circumstances may offer a new method for studying the mechanism of bronchial asthma.

Immunological Studies of Pollinosis. Mary Hewitt Loveless, M.D., New York, N. Y. (Abstract.)*

PART 1. THE RELATIONSHIP BETWEEN CLINICAL RESISTANCE AND THERMOSTABLE ANTIBODY IN THE CIRCULATION

The failure of others to find a correlation between the degree of clinical relief and the amount of thermostable or "blocking" antibody in the circulation of treated ragweed-sensitive patients may be due to the fact that the antibody requirement for different patients is not the same. When fifty-two patients with autumnal hay fever were observed over a three- to five-year period, the blood-borne antibody associated with a given degree of clinical resistance was found to vary considerably with different individuals. When comparisons were made between the concentration of humoral antibody and the associated degree of clinical resistance of *individual* patients over a succession of pollinating seasons, it was found that a close parallel existed between the amount of blood-borne antibody and of clinical immunity in 79 per cent of the patients, a fairly close parallel in another 19 per cent, and a lack of correlation in only one case.

The fluctuations in antibody titer were especially marked in eight members of the group whose injections of ragweed extract were replaced by physiologic saline solution during one or more years subsequent to their initial courses of treatment. After specific therapy had been discontinued, five of these patients lost two-thirds or more of the concentration of humoral antibody they had acquired as the result of subcutaneous injections of pollen extract. With this limited degree of immunity, their symptoms returned in undiminished intensity. The other three patients continued to produce a moderate amount of antibody despite the absence of treatment. As a result, two of them re-

*In press, Journal of Immunology.

ported they were "fairly comfortable" during the season in question; the third one had an "excellent" result.

The observations lead one to conclude that (1) thermostable antibody plays a protective role in pollinosis; and (2) the antibody concentration associated with a given degree of clinical immunity varies with the individual, a fact which must be taken into account in evaluating the role of the antibody.

It is suggested that the presumably protective influence of the antibody be put to test by allergists, who can roughly estimate the patient's tolerance before, during, and after a series of injections by means of the simple "quantitative" ophthalmic test, or the slightly more complicated method of testing the skin quantitatively. Fluctuations in the threshold of reaction of the conjunctiva to pollen extract vary in the same direction as the concentration of the antibody in the serum. "Quantitative" tests performed on the patient's skin likewise show these fluctuations in the threshold of sensitivity, more antigen being required to give the threshold response when the patient is undergoing active treatment than before or after, presumably due to the presence in the skin of relatively much or little thermostable antibody which is capable of neutralizing ragweed antigen.

Part 2. The Accelerated and Enhanced Immune Response in Hay Fever Patients*

If thermostable antibody against pollen plays a protective role in pollinosis, then it becomes important to determine optimal means of producing it. We have already shown that normal individuals react more quickly and strongly to secondary or "booster" doses of ragweed extract than to an initial course of injections. In the present report, it will be demonstrated that this enhanced and accelerated immune reaction occurs in pollen-sensitive patients when a primary series of injections is followed by a rest period and a secondary course.

By means of passive transfer tests, the amount of thermostable antibody present in the sera of fifty-two patients was determined before, during, and after treatment with ragweed extract. The clinical response to these injections was judged by the patient's general estimate of improvement and by his daily record of hay fever. The immunologic and clinical responses to the initial series of injections were then compared with those to subsequent courses given preseasonally in successive years.

Comparison Between Antibody Response of First and Subsequent Years.—When the same total dose of ragweed extract was given a patient in initial and in secondary courses, he was able to take the dose in a shorter time and to develop a larger amount of humoral antibody after he had been "educated" by the primary injections. This was demonstrated by twelve patients who received 1,000 units initially.

*From the Allergy Clinic of the New York Hospital, and the Department of Medicine of Cornell Medical College, New York, N. Y.

When the same dose was again given in the second year, they developed an average of three times as much antibody and took the course in half the time originally required. Numerous other patients, studied after a total dose of 5,000 units, 10,000 units and 50,000 units, showed the same tendency to take the dose faster and respond with greater antibody formation in a secondary series.

Practical Application of the Principle: Shortening the Treatment Period for Preseasonal Immunization.—The phenomena of accelerated and enhanced immune responses have been put to practical use. By knowing the patient's antibody requirement for clinical immunity and by determining the minimal dose which will produce this amount of antibody, it was possible to shorten the treatment period, markedly, in many instances. Forty-five of our patients, whose immunological response and requirement had been previously determined, were separated into three treatment-groups: the first to receive a total of only 1,000 units; the second, 10,000 units; and the third, an average of 32,000 units. The dose selected had been shown in past years to be adequate to produce a protective level of antibody for the patient concerned.

As might have been predicted, the clinical results were most gratifying and were comparable for the three groups. Not only was the dosage decidedly less than that given by allergists generally, but the time required for its administration was small. Three members of the first group, for example, took their complete course in one office visit, the 1,000 units being taken in three doses half an hour apart. The average time required for treatment in the first group was seventeen days; in the second group, thirty-six days; and in the third group, fifty-six days. Since ten days of rest were allowed preceding each bleeding, the actual time needed for injection was not more than five weeks in any case. It is a common practice to treat patients preseasonally for three to five months each year.

In summary, it has been found that practically all pollen-sensitive patients respond more quickly and more effectively to "booster" doses of pollen extract given after a six- to ten-months' rest period than to the initial series of injections. By titrating the thermostable antibody in the serum (or tissues) and correlating this with the clinical response, it is possible to know, roughly, how small a dose will produce a protective amount of antibody in a given patient. In some instances, a very short treatment will serve to produce excellent clinical immunity.

It is suggested that the simple "conjunctival" test be used to determine the patient's immunity, at intervals, during his primary course of injections, which should be intensive and carried out over a period of months; that subsequent treatment be based on the immunity acquired by the eye. It will be found in most cases that far less time and antigen will be required to immunize the patient in courses given subsequent to the first year's therapy. The protective dose must be determined for each individual.

Selected Abstracts

Immunology

Under the Direction of M. Walzer, M.D., and
Murray Albert, M.D., Brooklyn

Production of Local Skin Reactivity by Passive Transfer of Anti-Protein Sera. Chase, M. W.: Proc. Soc. Exper. Biol. & Med. **52**: 238, 1943.

Sera prepared against proteins were tested for their ability to produce local skin sensitivity in guinea pigs by passive transfer. Guinea pigs were actively sensitized to horse serum or ragweed pollen, usually with the aid of adjuvant emulsions of the Freund type. Skin sites were prepared on normal guinea pigs with 0.15 to 0.05 c.c. of selected antisera. After an interval of ten hours to several days, the sensitized sites were tested directly or by subcutaneous injection of the related antigen. Reactions consisting of erythema and edema were noted, the degree of reaction varying with the serum and with the particular recipient. The erythema usually diminished in less than an hour, while the edema increased for some time before subsiding. Following a subcutaneous injection of the related antigen, the sensitized sites reacted within ten to thirty minutes. Human reaginic sera (ragweed) failed to sensitize guinea pig skin. Precipitin sera produced in rabbits also locally sensitized normal guinea pig skin, and yielded large skin reactions when the related antigen was injected into the site. The rabbit immune sera employed were prepared with horse serum, hen ovalbumin, "picryl ovalbumin," and pneumococcus III polysaccharide. The skin of normal rabbits could not be passively sensitized with rabbit or guinea pig immune sera. It was suggested that the local skin reactivity which had been passively transferred, was probably mediated by anaphylactic antibodies.

Studies on Antibody-Antigen Mixtures. II. The Effect on Normal Living Excised Tissue and Its Dependence on the Presence of Free Antibody in the Mixture. Kulka, Anna M.: J. Immunol. **46**: 235, 1943.

In a previous paper, the author reported that the addition of antibody-antigen mixtures to Dale baths produced contractions of uterine

horns of normal guinea pigs. It was assumed that the antibodies became fixed to the cells and then reacted with the antigen. The present experiments confirmed this hypothesis. Type III rabbit antipneumococcal serum was mixed with Type III polysaccharide in such proportions that the further addition of antigen to the supernate failed to produce a precipitate, indicating the absence of free antibodies. To a Dale bath containing a normal uterine horn were added fresh normal guinea pig serum (complement), then supernatant serum, and finally Type III polysaccharide, in increasing amounts. No contractions resulted. The addition of fresh antiserum to the bath then produced a contraction. A greater amount of antiserum was required than in the control experiment, because an excess of antigen was present in the bath. In similar experiments, no contractions were obtained when the precipitate in saline solution was added to the bath in place of the supernate. In other experiments, the uterine horns in the Dale baths were treated with antiserum alone or a mixture of antiserum and small amounts of antigen. The bath was drained off within five minutes, and antigen was added in increasing amounts. Contractions were observed in almost every case, indicating that antibodies had been fixed to the tissue.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Incidence of Vasomotor Rhinitis During Pregnancy. Mohun, M.: Arch. Otolaryng. **37**: 699, 1943.

Mohun reports his observations on a group of twenty patients with vasomotor rhinitis selected from 1,900 obstetric patients. He states that vasomotor rhinitis and associated conditions have a definite incidence during pregnancy. Eight of the twenty patients experienced a severe degree of nasal blocking and congestion during pregnancy, while in the nonpregnant state none of these women had ever experienced any such trouble. Twelve other pregnant women experienced severe nasal blockage, but these patients had a history of previous transient attacks of hay fever and nasal blocking in the nonpregnant state. In nine patients with purulent sinusitis the infection was more protracted and the mucous membrane more swollen than is usually encountered. He concludes that the incidence of vasomotor rhinitis during pregnancy appears to parallel, and to be caused by, the amount of estrogen produced in the body. Typically, the condition disappears spontaneously after delivery.

Unilateral Vasomotor Rhinitis Due to Interference With the Cervical Sympathetic System. Fowler, E. P., Jr.: Arch. Otolaryng. **37**: 710, 1943.

Fowler reports the occurrence of unilateral vasomotor rhinitis due to interference with the cervical sympathetic system in a patient who had had a resection of the stellate ganglion and had developed a typical Horner's syndrome on the left side. Along with this developed a typical one-sided vasomotor rhinitis. Eosinophiles were demonstrated in the secretions. Four other patients with Horner's syndrome were observed. One with a condition of ten years' duration claims to have had blockage and watery secretion from one side of his nose after resection of the cervical sympathetic fibers for peripheral vascular disease. Three other patients with Horner's syndrome had no nasal symptoms. Fowler feels that further studies of the sympathetic system may throw some light on the cause of nasal allergy. In the first case of the patient with eosinophiles in the nasal secretions, it is possible that the allergy could have existed before the removal of the stellate ganglion.

A Note on the Evaluation of Privine HCl as a Nasal Vasoconstrictor. Fabricant, N. D., and Van Alyea, O. E.: Am. J. M. Sc. **205**: 122, 1943.

Fabricant and Van Alyea report studies on privine HCl, a new imidazoline derivative, 2-naphthyl-methyl imidazoline. It is crystalline and is readily soluble either in water or in normal saline. In experiments on animals it was found that a 0.1 per cent solution of privine HCl has no detrimental effect on ciliary activity. Intranasal injections in normal persons do not alter the pH of the nasal secretions. Privine HCl caused no irritation of the local mucous membrane and none of the toxic side effects sometimes observed with ephedrine.

Paredrine Hydrobromide-Sulfathiazole Therapy of Infections of Upper Respiratory Tract. Sulman, L. D.: Arch. Otolaryng. **37**: 713, 1943.

Sulman studied a group of seventy-five patients with rhinitis and sinusitis, over a period of eight months, in whom a suspension of sulfathiazole in an aqueous solution of paredrine hydrobromide had been used. He found this preparation effective in reducing nasal congestion and in decreasing or eliminating discharge without evident injury to the cilia. No untoward effects of any kind were observed. He further states that its use has shortened the course of infection for many patients and averted sequelae to colds.

Influence of Avulsion of the Trigeminal Nerve on the Human Nose. Lederer, F. L., and Dinolt, R.: Arch. Otolaryng. **37**: 768, 1943.

Lederer and Dinolt report some very interesting observations on the nose of patients in whom the trigeminal nerve had been resected. The

nasal history, the observations on the nose, and the results of biopsy of tissue taken from the turbinates of ten patients in whom the sensory root of the trigeminal nerve had been sectioned from five months to thirty-nine years previous to this investigation, were reported. No major changes were noted in pathology. Histologic study failed to reveal any differences between the two sides of the nose except for a possible decrease of mucoid degeneration in the nasal epithelium on the side of operation. Neither the vessels nor the glands evidenced changes which could be attributed to the lack of trigeminal fibers, nor was there a perceptible change in nasal secretion, ulceration, or crust formation.

Correspondence

The Editor,

The Journal of Allergy,

It has only recently come to my attention that Mr. O. C. Durham had a letter published in the JOURNAL OF ALLERGY for January, 1942, criticising an article by me published in September, 1941, on pollen grains (Alford, Ralph.: Pollen Studies, J. Allergy **12**: 572, 1941).

The technical part of this work was done by a well-known microscopist, Mr. Rolland Jenkins, and this was so stated in my article.

Inasmuch as Mr. Jenkins is better qualified to answer Mr. Durham's remarks, and inasmuch as my present position in the Army makes it difficult to answer without considerable delay and correspondence, it is fitting that you publish the enclosed letter from Mr. Jenkins.

CAPTAIN RALPH I. ALFORD.

May 10, 1943.

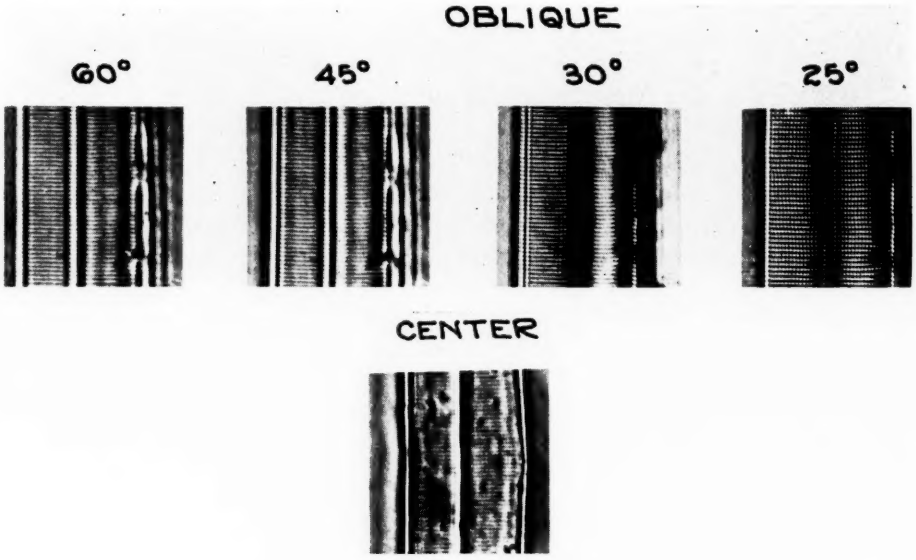
Dear Captain Alford:

In reading Mr. Durham's comments, it appears to me he is talking about something aside from the point you were making. In other works, he speaks about the *true* color of pollen and spores whereas you were seeking structural detail rather than color. He then says the dark-field method causes many details to be lost which could easily be seen by transmitted light. I do not agree, in fact I should say that a dark field emphasizes detail in many cases, and the wide use of dark field with unstained specimens (blood and microorganisms) is evidence of its further advantage, that is, the ability to see specimens in their natural form without the inevitable changes due to staining, etc.

It is not necessary to use Rheinberg filters at full aperture, and, in any event, the irradiation of transmitted light even with a decreased aperture causes definite loss of eye sensitivity to fine detail. Then there is the additional advantage of oblique illumination. The enclosed photos show very effectively the benefit of this. Here is one view with direct central illumination, with others at various degrees of obliquity.

Your arrangement of "tandem" eyepieces was admittedly "make-shift" for the definite purpose of availing of the greater depth of focus of the low-power objective. The arrangement is unorthodox and, therefore, subject to criticism from conservative microscopists, but the finest results I have ever seen as regards detail of relatively "thick" specimens was with *tandem microscopes,* which is certainly a departure from standard practice.

As regards magnification, you were magnifying an image at the focal (eye) point of the first eyepiece. If that image was 150x the specimen, and you magnified the image 5x, the resultant image was 750x the original. If you had done this with a 25x eyepiece alone, you would have had empty magnification. I have, by certain devices, secured photographic



images of 6,000 diameters which showed very good detail, but this can never be done with direct observation.

Finally, as regards the "reversed image" from the second ocular, all I can say is, "What's the difference? Turn your photo upside down."

ROLLAND JENKINS.

Montclair, N. J., May 6, 1943.

Editorial Announcement

Previously it has been customary to end the volumes of THE JOURNAL OF ALLERGY with the September issue. A change has been made, however, by which the future volumes will conform to the calendar year, beginning with the January issue and ending with that of November. The present Volume 14 will, therefore, continue through the November number, and the index which has been omitted from this number will appear instead in that issue.

Editor.